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VOLUME 11 DECEMBER, 1934 NUMBER 6

STUDIES ON THE NATURE OF RUST RESISTANCE IN WHEAT

VI. EFFECT OF HYDROGEN ION CONCENTRATION, PHENOLIC COMPOUNDS, AND HOST EXTRACTS ON THE GERMINATION OF UREDINIOSPORES OF *PUCCINIA GRAMINIS TRITICI*, FORM 21[1]

BY J. ANSEL ANDERSON[2]

Abstract

Urediniospores of *Puccinia graminis tritici*, form 21, were sown on buffer solutions covering the range from pH 3 to pH 8. Maximum germination was obtained between pH 5.8 and 6.5, with indications that if the point of maximum germination could be determined with precision it would be found to lie in the neighborhood of pH 6.2.

The effect of buffered and unbuffered solutions of pure phenolic compounds on the germination of urediniospores was studied. Greater inhibition was obtained with unbuffered solutions owing to the additional effect of hydrogen ion concentration. In buffered solutions, at pH 6.0, germination was prevented by 45 p.p.m. of hydroquinone, 125 of *o*-cresol, 180 of anisic acid, 250 of benzoic acid, 340 of catechol, 400 of guaiacol and 600 of phenol. A number of other compounds were also studied. The inhibitory effects of compounds, of which the bactericidal effects are known, are fairly closely related to their bactericidal efficiency.

Investigations were made of the inhibitory effects on spore germination and the growth of germ tubes, of aqueous dilutions of 15, 10, 5 and 2.5% of the press-juice of Vernal, Khapli, Marquis and Little Club wheats. Statistical treatment of the data accumulated in ten series of determinations, in which a total of 160,000 spores was counted, showed that significant differences occurred between varieties and that variations in the total solid content and hydrogen ion concentration of the press-juice had no consistent effect upon the results. Although the results were not entirely clear-cut, it is concluded that the varieties fall in the following order with respect to increasing inhibitory effect of their extracts: Vernal, Marquis, Khapli and Little Club. Since Vernal and Khapli are resistant to form 21 whereas Marquis and Little Club are susceptible, the results fail to show any relationship between the effects of host extracts on the germination of urediniospores, or on the growth of germ tubes, and the rust resistance of the variety.

Introduction

It is generally believed that, with certain exceptions, seedling resistance to *Puccinia graminis tritici* is physiological rather than morphological in nature. Susceptibility and resistance appear to depend upon a balance or lack of

[1] *Manuscript received August 9, 1934.*

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa, Canada, in co-operation with the Department of Field Crops, University of Alberta, Edmonton. Presented by R. Newton, F.R.S.C., at the meeting of the Royal Society of Canada, May, 1934.

[2] *Biologist, National Research Laboratories, Ottawa.*

balance between the physiological processes of the host and of the parasite. The complicated relations which exist between the various wheat varieties and biologic forms of the fungus preclude the possibility of any simple explanation of the differences between resistant and susceptible hosts. It seems probable that differences exist in the physiology of both the wheat varieties and the rust forms, and that the combinations and permutations of these give rise to the complicated relations which exist between host and parasite. If this be true, no explanation of the basis of rust resistance can be expected until adequate studies have been made of the physiological processes of a wide range of susceptible and resistant wheat varieties, and also of a large number of biologic forms of the parasite.

The difficulties of the investigation are increased enormously by the fact that the fungus cannot be cultured on artificial media. The spores will germinate and produce germ tubes, but growth is sustained for a day or so at most. In these circumstances it has not yet been possible to devise any adequate means whereby the effect on the parasite of any nutrient or growth inhibiting substances, which might be isolated from the host, can be studied.

Investigations which might have led to the development of such a test have been reported by Newton, Lehman and Clarke (7). They attempted to induce resistance in susceptible wheats by administering preparations of resistant varieties in three ways: (*a*) by adding them in various concentrations to water cultures of wheat seedlings; (*b*) by adding them to petri-dish cultures of wheat leaves; (*c*) by direct injection into leaves of plants growing in ordinary soil. Though the last two methods yielded indications of induced resistance, no clear-cut results were obtained.

Since that time Ezekiel (2) has published the results of an investigation designed, in part, to determine the relation between the ability of very dilute host extracts to support the growth of the germ tubes of urediniospores and the resistance of the host. The experiments, bearing directly on this discussion, were carried out mainly with Kanred and Mindum wheats and rust forms 18 and 19, to which the two wheats react reciprocally. In one experiment Little Club and form 1 were used in addition. In a further experiment 10 rust forms were tested, using extracts from Mindum wheat only. The urediniospores were cultured in hanging drops of 1% agar and a concentration of 0.25, 0.1, 0.01, 0.001 or 0.0001% of the host extract. Comparisons were made by measuring the length of the germ tubes after full growth had been attained. Interpretation of the results was complicated by the fact that the length of germ tubes depended upon the physiologic form used, the number of spores per unit volume of culture and the sample of spores used. Corrections were made for these three factors. The author concludes that: "Extracts prepared from wheat varieties differed in their ability to support the growth of physiological forms of *P. graminis tritici* in exact agreement with the respective resistance or susceptibility of the varieties to the various forms".

A careful study of the paper shows that the original data are extremely variable and that the methods used for correcting these for purposes of comparison are subject to serious objections. Nevertheless, the results appear to lend some support to the author's hypothesis.

Ezekiel's work appeared to open up possibilities for development of a test for the toxicity of host extracts which might be of considerable value as an adjunct to investigations of chemical differences between susceptible and resistant wheat varieties and their possible relation to rust resistance. In view of the variability of the results obtained by Ezekiel, and of the comparatively small differences between varieties, it was apparent that if conclusive results were to be obtained, further investigations would have to be made on such a scale that a body of data suitable for statistical analysis would be obtained. In these circumstances the highly developed and time-consuming technique of Ezekiel's germ-tube-measuring method precluded its use.

The alternative method of using more concentrated host extracts and measuring their inhibitive effect on spore germination rather than on the growth of germ tubes suggests itself at once. The method has certain objections. In the first place, it must be borne in mind that results obtained by germination counts cannot be directly compared with those obtained by measurements of germ tube length until it is shown that the inhibition of spore germination by higher concentrations of a compound or extract is directly related to the effect of lower concentrations on the growth of germ tubes. Thus similar results obtained by germination counts might serve to confirm Ezekiel's hypothesis, but contradictory results would not disprove it. However, since spores germinate on, and enter, the leaves of all wheat varieties (with certain possible exceptions due to the size and mechanism of stomatal openings), irrespective of whether the variety is resistant or susceptible, neither germination counts nor measurements of the growth of germ tubes can be said to simulate the conditions under which the fungus develops or fails to develop in the growing wheat plant. Thus there appears to be no *a priori* reason why one method should show a relation between the effect of extracts and varietal resistance and the other method should not.

In the second place, as early as 1902, Ward (10) reported that spores germinated equally well in extracts of the leaves of their own host species and of their antagonistic host species. This did not augur well for further investigations of spore germination. However the literature does not appear to contain any report of an investigation of spore germination on host extracts carried out on a scale capable of demonstrating definitely that varieties could be differentiated by this method. Such an investigation appeared to be worth undertaking.

The investigation was confined to studies of the effect of extracts of Vernal, Khapli, Marquis and Little Club wheats on the germination of urediniospores of a single rust form, No. 21. Preliminary experiments were made in 1932

on wheats grown in the field. Owing to the variability of the plants from day to day and to the fact that an insufficient number of determinations were made, the results obtained failed to demonstrate significant differences between varieties. However, the experience gained made it possible to plan the investigations carried out in 1933 which are reported in this paper.

Since Hursh (4) reported that the range of hydrogen ion concentrations over which maximum germination is obtained, differs for different rust forms, it was necessary to study the effect of hydrogen ion concentration on the germination of form 21 in order to determine what part this factor might play in the effect of host extracts.

The effect of dilute solutions of certain pure phenolic compounds upon the germination of urediniospores of form 21 was also investigated. It was hoped that time would be available for the study of the effect of a number of different compounds upon several rust forms. Since it has been shown that different bacteria differ in their resistance to different phenolic compounds (5) and that quite remarkable differences may exist in the resistance of different strains of the same bacterium (8), it seemed quite possible that various rust forms might show similar differences. If this proved to be true, the evidence accumulated might shed some light upon the characteristic ability of rust forms to attack certain wheat varieties and not others. Unfortunately it was not possible to complete this program and only a few preliminary studies of the reaction of spores of form 21 to phenolic compounds are reported.

Effect of Hydrogen Ion Concentration on the Germination of Urediniospores of Form 21

Materials and Methods

A pure strain of *Puccinia graminis tritici*, form 21, was obtained from the Dominion Rust Research Laboratory, Winnipeg. Stock cultures were maintained on Marquis seedlings grown in the greenhouse. No other rust forms were being cultured in this greenhouse at that time.

Clark and Lubs' (1) buffer solutions were used as a series of substrata covering the desired range, *viz.*: pH 3.0, phthalate and hydrochloric acid mixture; pH 4.0 to 6.2, phthalate and sodium hydroxide mixtures; pH 5.8 to 8.0, potassium acid phosphate and sodium hydroxide mixtures.

Spores from pustules four days old were used throughout the study, since preparatory investigations showed that spores from pustules of this age germinated most readily. Leaves bearing pustules were held over a dry petri dish and gently tapped. The spores thus collected were thoroughly mixed and a sample transferred to 5 ml. of the substrate, held in a syracuse dish, by dipping a camel-hair brush into the spores and tapping it above the dish. With practice a fairly even distribution of spores was obtained by this method. The dishes were stacked to prevent evaporation and incu-

bated in the dark for 15 hr. at 20° C. Germination counts were made under the microscope. In 1932 the determinations were carried out in duplicate, 500 spores being counted in each dish. In 1933 the determinations were carried out in quadruplicate and 250 spores were counted in each dish.

Experimental Results

The data obtained in 10 experiments carried out during 1932 and 1933 are reported in Table I, and a graph in which the mean percentage germination is plotted against pH is shown in Fig. 1. It was observed during the course of these experiments that the length of the germ tubes was roughly proportional to the number of spores germinating.

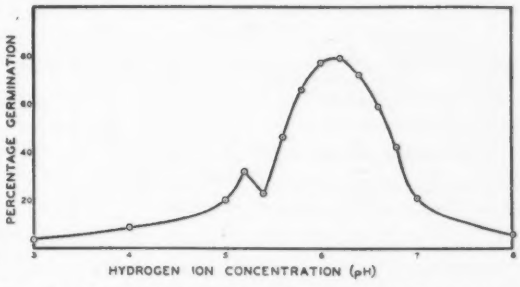

FIG. 1. *Effect of hydrogen ion concentration on the germination of urediniospores of form 21.*

Since Clark and Lubs' buffer solutions contain equal quantities of phthalate or phosphate, as the case may be, differences in pH being obtained by varying the concentration of acid and alkali, it seems reasonable to suppose that results obtained with these solutions will reflect only the effect of hydrogen ion concentration.

TABLE I

EFFECT OF HYDROGEN ION CONCENTRATION ON GERMINATION

Substrate	pH	May 29, 1932, %	1932, %	1932, %	1932, %	May 29, 1933, %	June 22, 1933, %	May 31, 1933, %	June 1, 1933, %	June 28, 1933, %	July 19. 1933, %	Mean %
Phthalate + HCL	3.0	9	5	—	—	0	1	—	—	—	—	4
Phthalate + NaOH	4.0	18	15	—	—	1	3	—	—	—	—	9
Phthalate + NaOH	5.0	31	37	36	29	6	13	5	8	8	29	20
Phthalate + NaOH	5.2	—	—	49	26	—	—	8	15	41	50	32
Phthalate + NaOH	5.4	—	—	64	50	—	—	4	3	0	18	23
Phthalate + NaOH	5.6	—	—	66	37	—	—	40	67	29	34	46
Phthalate + NaOH	5.8	—	—	92	69	—	—	61	69	44	62	66
Phthalate + NaOH	6.0	71	85	91	82	65	79	67	82	77	70	77
Acid phosphate + NaOH	6.2	—	—	87	84	—	—	83	72	77	70	79
Acid phosphate + NaOH	6.4	—	—	84	68	—	—	67	79	68	68	72
Acid phosphate + NaOH	6.6	—	—	72	71	—	—	48	60	58	46	59
Acid phosphate + NaOH	6.8	—	—	63	47	—	—	34	40	40	27	42
Acid phosphate + NaOH	7.0	33	37	40	27	8	10	25	16	7	11	21
Acid phosphate + NaOH	8.0	9	8	—	—	4	1	—	—	—	—	6

Each percentage represents the count of 1000 spores.

Statistical Analyses

The data obtained in the six series of determinations covering the range from pH 5.0 to pH 7.0 were examined statistically by the method of analysis of variance. The total variance of the observed results was analyzed into portions due to differences in the general level of the results obtained in different series, to the average difference over all series between hydrogen ion concentrations, and to the remainder, representing differences in the relative percentage germination at various hydrogen ion concentrations in different series. The results of the analysis of variance are given in Table II.

TABLE II

ANALYSIS OF VARIANCE OF DATA ON THE EFFECT OF HYDROGEN ION CONCENTRATION ON GERMINATION

Variance due to	Degrees of freedom	Sum of squares	Mean square
Series	5	6225	1245††
H ion concentrations	10	32324	3232††
Remainder	50	5455	109

†† *Significantly greater than the mean square due to remainder.*
In tables of analysis of variance throughout this paper
* *or* † *denotes that the mean square attains a 5% level of significance,*
** *or* †† *denotes that the mean square attains a 1% level of significance.*

The results show that there were significant differences in the general level of the results obtained in different series. This is attributed to differences in the spores used in different series. This portion of the variance, since its effect is equal at all hydrogen ion concentrations, may now be eliminated in further considerations of the data.

The average difference between hydrogen ion concentrations is shown to be highly significant. In order to evaluate the individual differences between the mean germinations for any two hydrogen ion concentrations, a criterion was set up as follows. The standard deviation of the mean of six determinations was calculated from the mean square due to the remainder and this multiplied by $\sqrt{2}$ and a factor gives the smallest difference between two means which can be judged significant for a 5% level of significance. The factor was obtained from published tables of "*t*" (3), the ratio of the observed difference between two means to the estimated standard error of the difference. The necessary difference for a 5% level of significance was found to be 12%. With this criterion in mind it is possible to evaluate the results of the investigation. It seems safe to assume that all hydrogen ion concentrations which give a percentage germination less than 67% (79%, the maximum germination obtained, minus 12%) fall outside the range of maximum germination. The range of maximum germination is therefore pH 5.8 to 6.5. The results also indicate that if the point of maximum germination could be determined with precision, it would be found to lie in the neighborhood of pH 6.2.

From the point of view of these studies the dip in the curve at pH 5.4 is of academic interest only. According to the criterion of the necessary difference, the mean percentage germination for pH 5.4 is not significantly lower

than that for pH 5.2. However, inspection of the data will show that the low point occurs as a result of the four series of determinations made in 1933. It therefore seems just to examine the significance of this low point by analyzing only these four series. Application of the "t test" to the differences between the means of the percentage germination at pH 5.2 and 5.6 and the percentage germination at pH 5.4, in these four series, gave a value of t equal to 6.47. Since the required value for a 1% level of significance is only 5.84 it appears that the drop in the percentage germination at pH 5.4, found in the 1933 series, is definitely significant. No explanation for this apparent abnormality has yet been found.

The results obtained with form 21 do not support Hursh's hypothesis (4) that forms more limited in their host range are also more limited in their tolerance to extremes of hydrogen ion concentration. Hursh studied forms 11 and 27 and found that the former, which attacks a greater variety of hosts, had a much wider range of maximum germination with respect to hydrogen ion concentration, than had form 27. The investigations of form 21, which compares with form 11 in its host range, show that it has a still more restricted range of maximum germination than Hursh reported for form 27. It appears therefore that Hursh's hypothesis is unsound.

Effect of Phenolic Compounds on the Germination of Urediniospores of Form 21

Materials and Methods

Rust spores were cultured by the method described previously. The compounds used were pure. Most of them were obtained from the Eastman Kodak Company, others had been prepared in the laboratory.

Since hydrogen ion concentration has such a marked effect on the germination of the rust spores, and since solutions of the various compounds used differ in their hydrogen ion concentration, both buffered and unbuffered solutions of the compounds were studied. Solutions of the compounds and of Clark and Lubs' hydrochloric acid and phthalate buffer solution for pH 6.0 were made up at double the required concentration and equal quantities of each were mixed together.

Germination tests were carried out as described previously. The approximate concentration at which each compound prevented germination was determined by the method of trial and error. A number of concentrations about this point were then tested in an attempt to determine more accurately the exact concentration which would just prevent germination. The number of compounds which could be tested and the highest concentration at which they could be used were limited by the solubility of the compounds in water.

Experimental Results

A summary of the results is reported in Table III. For the more toxic and more soluble compounds, the concentration at which germination was completely inhibited is reported. In other cases the percentage germination is reported for the highest concentration tested.

TABLE III

EFFECT OF PHENOLIC COMPOUNDS ON GERMINATION

—	Buffered (pH 6)		Unbuffered		
	Conc., p.p.m.	Germ., %	Conc., p.p.m.	Germ., %	pH
Phenols					
Hydroquinone	45	0	15	0	5.7
Catechol	340	0	200	0	4.6
Guaiacol	400	0	—	—	—
Phenol	600	0	600	0	7.1
Pyrogallol	800	4	1600	0	6.2
Resorcinol	750	6	1200	0	5.1
Phloroglucinol	800	55	1600	47	5.0
o-Cresol	125	0			
m-Cresol	125	61			
Phloracetophenone	200	22			
Quercetin	400	34			
Quercitrin	400	34			
Phenolic acids, etc.					
Anisic acid	180	0	40	0	4.2
Benzoic acid	250	0	25	0	—
Salicylic acid	800	4	60	0	3.6
Protocatechuic acid	800	16	400	0	3.6
o-Methoxybenzoic acid	800	16	200	0	3.6
Vanillic acid	800	38	600	0	3.3
p-Methoxybenzoic acid	800	58	800	0	—
Gallic acid	800	68	800	3	3.4
β-Resorcylic acid	800	72	800	2	—
Aconitic acid	800	72	800	2	2.8
Syringic acid	800	88	800	0	3.3

DISCUSSION

The data reported must be considered as approximate, though they are probably sufficiently accurate for the purpose of placing the compounds in the order of their toxicity. The exact point at which each compound prevents germination is not readily determined. With some compounds germination falls off steadily as the concentration is increased, but with others the germination falls to about 5 to 10% and then decreases very slowly as the concentration is increased. In the latter cases the concentration at which germination is entirely inhibited is difficult to determine and may not adequately represent the toxicity of the compound.

It appears that if this investigation is extended to include the study of the effects of a number of compounds on different rust forms, the method used in the work described in this paper would not be suitable. Repetition of a series of simultaneous determinations of the effects of a number of different concentrations of different compounds on the various rust forms and statistical analyses of the data would probably yield more accurate results.

The inhibitory effect of the compounds, of which the bactericidal properties have already been studied (5, and papers cited therein), on the germination

of urediniospores of form 21, is fairly closely related to their bactericidal properties. Because of the small number of compounds of the same category tested, no attempt can be made to offer a hypothesis relating chemical constitution to inhibitory effect. The possible importance of the positions of two similar groups in the nucleus is amply illustrated by the differences in toxicity between *o*- and *m*-cresol and between catechol, resorcinol and hydroquinone. In this connection, however, it must be pointed out that physical properties may be more important than chemical ones in determining the toxicity of the compounds.

During the course of these studies it was observed that concentrations of certain of the compounds, of such strength that germination was scarcely affected, stimulated the growth of the germ tubes. This effect was particularly marked with a solution of 200 p.p.m. of phenol in which the germ tubes attained a much greater length than those of spores germinated in water. This observation is in accord with our knowledge of the action of poisons, most of which are stimulative if taken in small enough doses. The observation is also of interest in connection with the results of comparisons of the effects of host extracts made by estimating the inhibitory effect on spore germination and on the growth of germ tubes; it is discussed further in a later section of this paper.

Effect of Host Extracts on the Germination and Growth of Germ Tubes of Urediniospores of Form 21

Materials and Methods

Four wheat varieties were used in these studies, namely, Vernal, Khapli, Marquis and Little Club. The first two varieties are resistant to form 21 and the last two are susceptible. Stakman and Levine (9) record the reactions as follows:

Variety	Range	Mean
Vernal	0 to 1	0;
Khapli	0 to 1	1=
Marquis	3++ to 4+	4
Little Club	3++ to 4++	4

The plants were grown in the greenhouse. Separate seedings were made for each series of determinations. The plants were harvested ten days after emergence by cutting them off just above the ground level. The press-juice was obtained by the method of Newton, Brown and Martin (6). It was centrifuged, decanted and diluted with distilled water to the required concentration. Harvesting and all subsequent operations were carried out for all four varieties as nearly concurrently as possible.

The press-juice of each variety was tested at four concentrations, namely, 15, 10, 5 and 2.5%. Four dishes were used for each concentration of each

variety and 250 spores were counted in each dish. Seeding, incubation, and counting of the spores were carried out in the manner previously described.

In planning the investigation no provision was made for measuring the length of the germ tubes, since to do this on a scale commensurate with the determinations of percentage germination would have required more time than was available. However, during the course of the investigations rough and rapid estimates of the length of the germ tubes were made. The mean length of the tubes in each dish was estimated by selecting two or three tubes, which appeared by inspection to be of average length, and measuring and averaging these. The method is open to serious objections from the statistical point of view and no great reliance can be placed upon the results.

The total solids of the undiluted press-juice were determined by means of the refractometer, and the hydrogen ion concentrations of the 15 and 2.5% dilutions were determined potentiometrically.

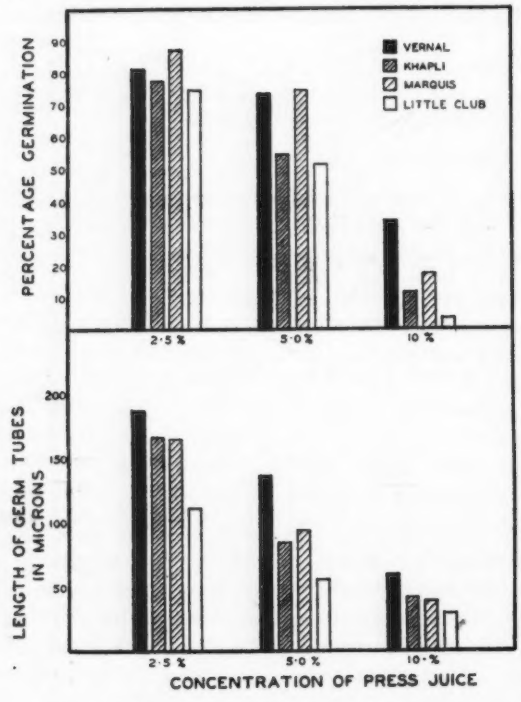

FIG. 2. *Effect of host extracts on (upper) percentage germination and (lower) growth of germ tubes of urediniospores of form 21.*

EXPERIMENTAL RESULTS

The results of ten series of determinations are given in Table IV. The values under percentage germination represent the mean of four counts of 250 spores; the values under length of germ tubes represent the mean of estimates of length made for each of four dishes. In order to facilitate examination of the data, they are given in condensed form in Table V as the mean for all varieties by series, and in Table VI as the mean for all series by varieties. The data on the percentage germination and on the length of germ tubes given in Table VI are also presented graphically in Fig. 2.

TABLE IV

EFFECT OF HOST EXTRACTS IN CONCENTRATIONS OF 15, 10, 5 AND 2.5% ON THE GERMINATION OF SPORES AND ON THE GROWTH OF GERM TUBES

Date, 1933	Variety	Total solids	pH		Mean percentage germination					Mean length, microns			
			15%	2.5%	15%	10%	5%	2.5%	0%	10%	5%	2.5%	0%
June 9	Vernal	6.2	6.23	6.29	0.2	4.1	55.8	67.1	87.0	—	98	153	330[1]
	Khapli	5.4	6.03	6.12	0.4	3.3	41.0	70.9		—	65	140	
	Marquis	6.2	6.22	6.26	0.3	23.0	62.6	84.6		—	88	153	
	L. Club	7.1	6.03	6.12	0.2	1.6	44.2	64.4		—	42	91	
June 13	Vernal	6.2	6.45	6.43	19.3	40.2	78.9	64.5	91.9	88	225	195	
	Khapli	5.8	6.34	6.32	0.7	23.5	72.5	76.2		39	143	192	
	Marquis	6.2	6.35	6.33	0.1	16.3	82.7	89.4		33	91	182	
	L. Club	6.6	6.36	6.33	0.1	5.3	53.9	84.4		26	46	130	
June 16	Vernal	6.2	6.29	6.37	18.9	66.1	89.2	92.5	87.7	96	183	225	
	Khapli	5.8	6.15	6.32	0.9	22.5	65.9	89.3		53	125	198	
	Marquis	5.4	6.30	6.33	0.6	24.9	83.6	87.7		38	85	171	
	L. Club	6.2	6.18	6.24	0.0	5.6	70.8	85.3		30	60	133	
June 20	Vernal	5.8	—	6.18	2.1	45.0	76.5	88.2	92.7	49	137	205	
	Khapli	6.2	—	6.16	0.2	5.9	67.6	87.4		39	55	150	
	Marquis	7.5	—	6.06	0.9	36.7	88.3	90.9		42	114	221	
	L. Club	6.2	—	6.15	0.0	1.4	72.8	89.2		—	65	101	
June 23	Vernal	5.8	6.50	6.65	0.0	1.9	72.5	92.8	92.0	—	65	173	
	Khapli	5.0	6.32	6.40	0.0	8.3	62.6	92.2		33	73	183	
	Marquis	6.2	6.26	6.37	0.2	9.7	74.9	95.5		38	68	145	
	L. Club	6.6	6.22	6.35	0.1	0.2	56.0	75.2		—	45	100	
June 27	Vernal	7.1	6.33	6.40	5.1	43.9	74.1	81.5	87.7	43	140	190	
	Khapli	5.0	6.15	6.27	0.2	10.7	51.3	76.9		35	90	160	
	Marquis	5.8	6.28	6.30	0.6	16.4	71.8	87.3		40	80	135	
	L. Club	6.6	6.18	6.34	0.0	2.6	38.7	67.8		30	40	125	
June 30	Vernal	5.4	6.20	6.30	19.6	46.3	58.5	80.6	94.6	50	120	245	
	Khapli	5.0	6.03	6.10	0.0	2.6	49.1	63.3		30	73	143	
	Marquis	5.4	6.30	6.30	0.0	6.1	59.1	77.5		30	65	153	
	L. Club	5.8	6.20	6.30	0.0	1.5	35.5	64.6		—	38	80	
July 4	Vernal	7.1	6.17	6.34	10.1	34.6	82.7	89.6	94.3	40	98	143	
	Khapli	5.8	6.00	6.05	7.0	10.3	57.6	88.1		38	78	178	
	Marquis	6.2	6.20	6.38	0.0	0.6	61.2	89.1		—	60	108	
	L. Club	—	6.00	6.09	0.0	0.5	40.0	87.0		—	60	110	
July 7	Vernal	7.1	6.20	6.40	0.8	19.4	65.7	73.0	91.8	30	138	175	
	Khapli	5.8	6.06	6.20	0.0	0.7	39.3	60.9		—	60	133	
	Marquis	5.4	6.00	6.30	0.2	12.4	79.1	85.8		—	125	185	
	L. Club	—	6.08	6.14	0.0	16.4	70.5	75.3		30	105	125	
July 12	Vernal	6.2	6.18	6.35	34.4	38.6	79.8	82.7	88.6	83	153	185	
	Khapli	5.4	6.00	6.32	2.0	26.2	35.7	71.8		65	88	195	
	Marquis	5.8	6.06	6.24	4.6	25.6	79.7	85.6		55	160	200	
	L. Club	7.5	6.06	6.30	0.0	1.7	30.1	52.6		—	63	128	

[1] *Average length of germ tubes of spores sown on water.*

TABLE V

EFFECT OF HOST EXTRACTS ON THE GERMINATION OF SPORES AND THE GROWTH OF GERM TUBES

(Means by days)

Date	Total solids	pH	Percentage germination					Length in microns			
			15%	10%	5%	2.5%	0%	10%	5%	2.5%	0%
June 9	6.2	6.17	0.3	8.0	50.9	71.8	87.0	—	73	134	330
13	6.2	6.37	5.1	21.3	72.0	78.6	91.9	47	126	175	
16	5.9	6.28	5.1	29.8	77.4	88.7	87.7	54	113	182	
20	6.4	6.14	0.8	22.3	76.3	88.9	92.7	(43)	93	169	
23	5.9	6.39	0.1	5.0	66.5	88.9	92.0	(36)	63	150	
27	6.1	6.29	1.5	18.4	59.0	78.4	87.7	37	88	153	
30	5.4	6.22	4.9	14.1	50.6	71.5	94.6	(37)	74	155	
July 4	6.4	6.16	4.3	11.5	60.4	88.5	94.3	(39)	74	135	
7	6.1	6.18	0.3	12.2	63.7	73.8	91.8	(30)	107	155	
12	6.2	6.19	10.3	23.0	56.3	73.2	88.6	(68)	116	177	

Brackets indicate mean of less than four varieties.

TABLE VI

EFFECT OF HOST EXTRACTS ON THE GERMINATION OF SPORES AND THE GROWTH OF GERM TUBES

(Means by varieties)

Variety	Total solids	pH	Percentage germination					Length in microns			
			15%	10%	5%	2.5%	0%	10%	5%	2.5%	0%
Vernal	6.3	6.33	11.0	34.0	73.4	81.3	90.8	60	136	189	330
Khapli	5.5	6.18	1.1	11.4	54.3	77.7		42	85	167	
Marquis	6.0	6.25	0.8	17.2	74.3	87.3		39	94	165	
L. Club	6.6	6.20	0.0	3.7	51.3	74.6		29	56	112	

STATISTICAL ANALYSES

Analyses of Variance of Percentage Germination and Length of Germ Tubes

The data on the percentage germination and the length of germ tubes were subjected to an analysis of variance. The data obtained by each method of estimating inhibitory effect were analyzed separately, and in both cases the results at each concentration were also examined separately. The variance of the observed results was analyzed into portions due to; (i) variations in the general level of the results obtained in different series, (ii) to the average differences, over all series, between varieties, (iii) to differences in the relative performance of the varieties in different series, and (iv) to differences between parallel counts. The results of these analyses are summarized in Table VII. No analysis is reported for the length of germ tubes in 10% concentrations of the press-juice as the data for these determinations were incomplete.

TABLE VII

ANALYSES OF VARIANCE OF GERMINATION AND LENGTH OF GERM TUBE

Variance due to	Degrees of freedom	Mean squares					
		Percentage germination				Length of germ tubes	
		15%	10%	5%	2.5%	5%	2.5%
Series	9	1074	5906	9393**	5960**	7640*	3848
Varieties	3	6821**	41450**	37443**	7493**	42848**	42334**
Series × Varieties	27	812††	3123††	2187†	1309††	3118††	2762††
Parallels	120	226	767	1176	539	816	1235

* *and* ** *Significantly greater than the mean square due to series × varieties and to that due to parallels.*

† *and* †† *Significantly greater than the mean square due to parallels.*

The analyses of variance show by the magnitude of the mean squares due to series and to the interaction between series and varieties, that there was considerable variation in the results from series to series. It is presumed that the main factors contributing to this are the variations in spores and wheat plants, and possible slight differences in technique.

The mean square due to series, since it represents that portion of the variation between series which affects all varieties to the same extent, may be eliminated from the comparisons of the data. That portion of the variation between series which affects the varieties differentially, that is, the interaction between series and varieties, is of considerable importance. The analyses show that the mean square due to the interaction was in all cases significantly greater than the mean square due to variance in the parallel counts. It is therefore the variance due to the interaction and not that due to parallel counts which is the main factor in reducing the accuracy of the comparisons of varieties.

Two factors which probably play a part in producing the differences in the relative performances of different varieties in different series suggest themselves: first, the variations in the total solids of the press-juice both within and between varieties over the ten series; and, second, similar variations in the hydrogen ion concentration of the press-juice. Investigations of the possible effects of these factors are described later.

Notwithstanding the comparative magnitude of the interaction effect, the main object of this investigation, the establishment of significant differences between varieties, was attained, as is shown by the fact that the mean square due to varieties is in all cases significantly greater than those due to the interaction and parallel counts. Or, in other words, in spite of the different relative performances of the varieties in different series, the average performances of the varieties, over all series, differed significantly. In order to facilitate the comparison of the varieties, the differences between the means for all series, for each pair of varieties, are given in Table VIII. The smallest differences

between the means, required for a 5% level of significance, were calculated from the mean squares due to the interaction by the method previously described. The values obtained are also given in Table VIII.

TABLE VIII

DIFFERENCES BETWEEN VARIETAL MEANS FOR ALL SERIES

Difference	Percentage germination				Length of tubes	
	15%	10%	5%	2.5%	5%	2.5%
L. Club – Marquis	− 0.8	**−13.5**	**−23.0**	**−12.7**	**−38**	**−53**
L. Club – Khapli	− 1.1	− 7.7	− 3.0	− 3.1	**−29**	**−55**
L. Club – Vernal	**−11.0**	**−30.3**	**−22.1**	− 6.7	**−80**	**−77**
Marquis – Khapli	− 0.3	+ 5.8	**+20.0**	+ 9.6	+ 9	− 2
Marquis – Vernal	**−10.2**	**−16.8**	+ 0.9	+ 6.0	**−42**	**−24**
Khapli – Vernal	− 9.9	**−22.6**	**−19.1**	− 3.6	**−51**	−22
Necessary difference for 5% level	5.2	10.3	8.6	6.6	26	24

Numbers in bold face are significant differences.

The differences were obtained by subtracting the value for the more resistant variety from that for the less resistant. A positive difference therefore indicates that the extract of the more resistant variety had a greater inhibitory effect, and supports the hypothesis that the inhibitory effect of host extracts is directly correlated with the rust resistance of the hosts. It will be observed that of the 36 differences reported in Table VIII, only 6 are positive; and that of the 23 differences which are significant, only 2 are positive. Further, if the effect of host extracts on spore germination or the growth of germ tubes had any relation to the rust resistance of the host, it seems reasonable to suppose that the varieties would have been divided into two groups; the resistant varieties, Vernal and Khapli, and the susceptible varieties, Marquis and Little Club, being paired. The results show no such pairing.

Investigation of the Possible Effect of Variations in Total Solids

The variance of the total solids of the press-juice was also analyzed; the results are given in Table IX.

TABLE IX

ANALYSIS OF VARIANCE OF TOTAL SOLIDS OF THE PRESS-JUICE

Variance due to	Degrees of freedom	Sum of squares	Mean square
Varieties	3	3.654	1.218
Series	9	4.632	.514
Remainder	27	17.742	.630

The mean square due to the varieties was not significantly greater than that due to the remainder. In other words, the relative quantities of total solids in the press-juice from the four varieties differed so much between series that it cannot be said that the means for the varieties, for all series, differ from each other significantly. In these circumstances it is apparent that the differences in total solids of the press-juice could have had no consistent effect upon the results of the main investigation.

Investigation of the Possible Effect of Variations in Hydrogen Ion Concentration

The data for the hydrogen ion concentration of 2.5% concentrations of the press-juice were also subjected to an analysis of variance; the results are given in Table X.

TABLE X

ANALYSIS OF VARIANCE OF HYDROGEN ION CONCENTRATION OF THE PRESS-JUICE

Variance due to	Degrees of freedom	Sum of squares	Mean square
Varieties	3	0.1318	0.0439††
Series	9	.2732	.0304
Remainder	27	.1476	.0051

†† *Significantly greater than the mean square due to remainder.*

The mean square due to the varieties proved to be significantly higher than that due to the remainder. The necessary difference between the means for varieties, for all series, calculated for a 5% level of significance, was found to be 0.05 pH units. The press-juice of Vernal has the lowest hydrogen ion concentration, pH 6.37, followed by that of Marquis, pH 6.29, with those of Khapli and Little Club, pH 6.23 and 6.24, highest.

Reference to the first part of this paper will show that the data obtained on the effect of hydrogen ion concentration on spore germination indicate that if the concentration at which maximum germination takes place could be determined with precision it would be found to lie in the neighborhood of pH 6.2. If hydrogen ion concentration had any considerable effect on the germination of spores in 2.5% concentrations of the host extracts, it would appear that Vernal and Marquis should have shown a greater inhibitory effect than Khapli and Little Club. The results show, on the contrary, that the reverse was true, thus indicating that hydrogen ion concentration was not an important factor.

In order to investigate further the possibilities of a significant correlation between percentage germination and hydrogen ion concentration, the data obtained with 2.5% concentrations of the press-juice were subjected to an analysis of variance and covariance. The relationship was assumed to be curvilinear and the percentage germination was correlated with both the pH and the square of the pH. The coefficients of regression of percentage germination on pH and on the square of the pH were calculated and their significance tested by determining the proportion of the total variance in germination capable of representation by the regression equation. The mean square due to the regression did not differ significantly from the mean square deviation, showing that there was no appreciable correlation between percentage germination and hydrogen ion concentration. It is concluded, therefore, that the variations in hydrogen ion concentration within and between varieties, and between series, had no consistent and significant effect upon the results of the main investigation.

In this connection, however, it is interesting to note that the results of a single series of determinations, carried out as an afterthought, in which the

press-juice was diluted with buffer solutions of pH 6.1, instead of with distilled water, did not agree entirely with the results of the main series of investigations. The data obtained are given in Table XI.

TABLE XI

THE EFFECT OF HOST EXTRACTS DILUTED WITH A BUFFERED SOLUTION ON THE GERMINATION AND GROWTH OF GERM TUBES OF UREDINIOSPORES

Variety	Mean percentage germination					Mean length, microns		
	15%	10%	5%	2.5%	0%	10%	5%	2.5%
Vernal	14.2	21.5	21.1	28.8	89.1	54	53	72
Khapli	3.1	7.5	28.2	32.5	—	19	71	81
Marquis	9.5	19.2	41.1	56.3	—	48	103	141
L. Club	0	5.8	36.4	35.5	—	15	91	89

It will be observed that at the lower concentrations, the varieties fall in a more logical order. That it would be unwise to place much weight upon the results of a single series is amply illustrated by the fact that the order of the varieties, as judged by the effect of 2.5% concentrations on the germination of spores, is exactly the same in the series carried out on June 13, diluting with water, as it is in the series under discussion. Unfortunately, owing to the incidence of hot weather and consequent irregularity in the germination of the spores, it was not possible to carry out further investigations of the effects of press-juice diluted with buffered solutions. The results of the single series involving this technique are included since they suggest possibilities for further investigations.

Relation between Percentage Germination and Length of Germ Tubes

The correlation coefficients between percentage germination and length of germ tubes for each variety were calculated and found to be as follows: Vernal 0.79, Khapli 0.88, Marquis 0.80, and Little Club 0.80. The scatter diagrams for the four varieties are shown in Fig. 3. The numerals represent the number of the series. Inspection of the diagrams will show that the correlations are not biased by differences in the general level of the results from series to series. There is, therefore, a high degree of correlation within varieties, between the two methods of estimating inhibitory effect.

The fact that the varieties did not fall in exactly the same order at different concentrations, when placed according to the effect of extracts on germination and on the growth of the germ tubes, suggests that the relation between percentage germination and length of germ tubes is not the same for all varieties. The scatter diagrams offer evidence in support of this conclusion. It will be observed that the slope of the regression line is not the same in all four diagrams. The numerical values for the slope are as follows: Vernal 2.51, Khapli 1.79, Marquis 1.59, and Little Club 1.11. In addition it will be observed that the relationship in the case of Marquis appears to be definitely

curvilinear whereas those for the other three varieties are almost linear. In these circumstances, it is not surprising that the two methods of comparing the inhibitory effect of extracts of different varieties did not yield exactly the same results at different concentrations.

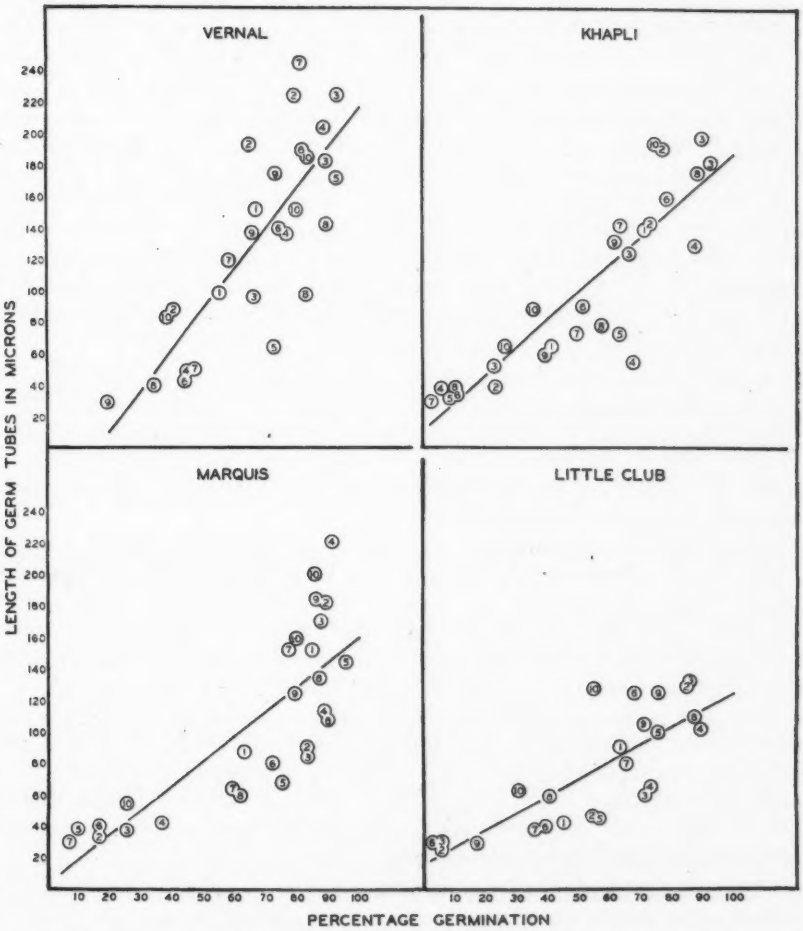

FIG. 3. *Correlation between the effects of host extracts on the germination of urediniospores of form 21 and on the growth of germ tubes.*

Discussion

The results of the investigation of the effects of host extracts on the germination of urediniospores and the growth of germ tubes are characterized by extreme variability. In these circumstances the interpretation of the results, even with the aid of statistical methods, is not easy. The statistical treatment

serves to divide the variance into a number of portions, *viz.*: a portion due to differences in parallel counts; a portion due to differences between varieties; a portion due to differences between series which affect all varieties to an equal extent; and a portion due to differences between series which affect the varieties differentially. The results also show that variations in concentration of press-juice exert a differential effect not only on different varieties but also on the germination and growth of germ tubes of the same variety.

It seems safe to assume that the variability in parallel counts is largely the result of sampling errors. These arise in several ways. The limiting error, in all probability, is the fact that the 250 spores counted, and more particularly the two or three germ tubes selected for measurement, do not constitute a representative sample. Additional errors may be introduced by the fact that a representative sample of the original spores may not be transferred to each dish and by the fact that the distribution of the spores in the dish also affects germination and growth. The results show, however, that the variability of the parallel counts was of comparatively little importance since it was by no means the limiting error of the determinations as a whole.

That part of the variation between series which affects all varieties to the same extent, though of considerable magnitude, is of little importance from the point of view of comparing varieties. These variations are probably attributable to differences in the conditions of the spores and wheat plants used in different series, presumably caused by variations in the environmental conditions in the greenhouse.

That part of the variation between series which affected the varieties differentially constituted the limiting error of the determinations. The results indicate quite definitely that the relative inhibitory properties of the extracts of different varieties differed from day to day. Since the plants of each variety, for any given series, were grown under conditions as nearly similar as possible, it seems reasonable to suppose that differences in the environmental conditions under which the plants of different series were grown affected their constitutions differentially, and thus had differential effects upon the inhibitory properties of their extracts. Evidence supporting this conclusion is offered by the fluctuation from series to series in the relative quantities of total solids in the press-juice of the different varieties, and by similar variations in hydrogen ion concentration.

The fact that variations in total solids of the press-juice had no consistent effect upon the results of the investigation is difficult to explain. It seems probable that the quantities of the various compounds in the press-juice varied independently and that measurement of total solids was therefore not a measure of the quantity of the inhibitive compound or compounds present in the extracts. It is also possible that physical properties not reflected by an estimate of total solids are important factors.

Differences in the relative inhibitory effects of the varietal extracts, at different concentrations, and on germination and growth, are not as important as might be thought at first glance. It is true that the order of the

varieties with respect to inhibitory effect differs for different concentrations, but reference to Table VIII will show that in no case was a variety shown to have a significantly greater inhibitory effect than another variety at one concentration and a significantly less effect at another. Neither did the results of one method of measuring inhibitory effect contradict those obtained by the other. The cases in which the order of the varieties is changed from one concentration to another, or between methods, are those in which the methods failed to differentiate the varieties.

In spite of the variations discussed above, both tests were sufficiently accurate to show that differences exist between the average inhibitory effects of extracts of certain of the varieties. When placed in order of increasing inhibitory effect on germination, the varieties fall in the following order: Vernal, Marquis, Khapli and Little Club. The germination test fails to separate Vernal and Marquis at the two lower concentrations, Marquis and Khapli at the two higher concentrations and Little Club and Khapli at all concentrations. When placed in order of increasing inhibitory effect on the growth of germ tubes, the varieties fall in the same order. This method fails to differentiate Marquis and Khapli. The results as a whole fail to show any relation between the effect of host extracts on the germination of urediniospores, or on the growth of germ tubes, and the rust resistance of the variety.

These conclusions do not agree with those of Ezekiel. On the other hand, they do not disprove his hypothesis. Ezekiel worked with such dilute extracts of the hosts that the growth of the germ tubes was increased over that of the controls. In the investigations reported in this paper, more concentrated extracts were used and the growth of the germ tubes was inhibited in all experiments with such extracts.

In the course of the investigation of the effects of phenol on the germination of urediniospores it was noted that a solution of 200 p.p.m., which had a comparatively slight effect on germination, stimulated the growth of the germ tubes remarkably. At increased concentrations the germination of the spores was reduced and the growth of the tubes inhibited. At a concentration of 600 p.p.m. no germination or growth took place. Ezekiel's results, together with those reported in this paper, suggest that host extracts behave similarly; that although they reduce growth at high concentrations they stimulate it at very low concentrations. However, there appear to be no adequate reasons for assuming that different varieties would fall in the same order when placed according to the inhibitory effects of their concentrated extracts on rust spores, and according to the stimulating effects of very dilute extracts.

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THE EFFECT OF POTASSIUM SUPPLY ON THE COMPOSITION AND QUALITY OF WHEAT[1]

By A. G. McCalla[2]

Abstract

The amount of minerals absorbed by wheat plants and the time of absorption influenced the quality of the grain produced. The quality was adversely affected by restriction of potassium supply, and this effect could not be overcome by supplying sodium in place of potassium. The quality of the grain produced by plants which had a small supply of potassium from germination until heading was better than that of grain from plants which absorbed the same amount of potassium in the first month of growth, but none from then on.

Introduction

In 1907, Wood (17) suggested that the acids and salts in the cell sap of wheat when the endosperm is forming impress upon the gluten the physical properties which decide the character of the flour obtainable from that wheat. This suggestion, in modified form, has been revived by Gericke (4, 5), who advanced the thesis that the variation in the quality of protein of wheat flour, as determined by baking methods, can be partially accounted for by colloidal phenomena expressing themselves in the physical properties of proteins and starches, the nature of these physical properties being determined by the absorption of certain inorganic elements in excess of the amounts required for the production of the crop. Experimentally, Gericke (4) found that better bread was obtained from wheats which had a relatively large excess of salts in the straw, beyond the minimum required for the yield obtained, than from those which had no excess or only a slight one.

More recently, Gericke (6) studied the effect of supplying maturing wheat plants with nitrate in combination with various cations. He demonstrated that the quality of the bread produced from these wheats varied with the nature of the cation of the salt used to supply the nitrogen, and concluded that the mineral nutrition of the wheat plants markedly affects the quality of the protein produced in the grain.

As the baking quality of flour is dependent to a large extent on the quality of the gluten laid down in the wheat grain, then, on the basis of Gericke's results, variations in the nutrition of the wheat plant will of necessity have an influence on the quality of the flour. In an earlier paper (11) the writer reported a detailed study of the effect of limiting the nitrogen supply on the composition and quality of wheat. The general conclusion reached was that, although the course of nitrogen metabolism in the vegetative parts of the plant was altered, and the total nitrogen content of the grain markedly influenced, the quality of the gluten produced in the wheat grain was not affected by limiting the supply of inorganic nitrogen.

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[2] *Research Assistant, Associate Committee on Grain Research, University of Alberta.*

Probably the availability of many elements and the physiological relations existing among them play important parts in determining the course of nitrogen metabolism. A survey of the literature reveals that potassium is considered essential in the early stages of this process, but there is far from unanimous agreement regarding the most important role of this element in the development of green plants. Hoagland (8) points out that researches on potassium functions are necessarily concerned with resultant effects of potassium deficiency on growth and metabolism. Although it is difficult to allocate the functions of potassium to specific steps in the whole chain of metabolic processes, recent literature indicates that increasing attention is being paid to nitrogen metabolism and cell division.

Ginsburg (7) studied the effect of omitting potassium from culture solutions in which soy beans were growing. He found that the total nitrogen content of the plants grown in a solution in which the lack of potassium was compensated for by calcium, was less than half, but the calcium content more than double, that of the check plants. When the lack of potassium was compensated for by sodium, the amount of total nitrogen absorbed was greater, and the amount of calcium slightly less, than where calcium was used.

Potassium-deficient soy beans were also studied by Burrell (2), who concluded that one important role of potassium was in the formation of proteins from carbohydrates and amino acids. This conclusion was based on the fact that the amount of insoluble nitrogen was less, but that of the non-protein fraction, particularly amino nitrogen, greater in potassium-deficient than in normal plants.

Burrell's conclusion was supported by the work of Nightingale *et al* (13) with potassium-deficient tomato plants. They concluded that potassium was probably essential for the synthesis of proteins in meristematic tissues, but probably not essential for later stages of storage protein synthesis.

These investigators also found that nitrate assimilation was retarded in potassium-deficient plants, and concluded that potassium was directly or indirectly essential in the initial stages of nitrate reduction. In the same publication, it is reported that Dr. Eckerson found that the nitrate reducing power of potassium-deficient beet and lettuce plants was much less than that of the check plants.

Loew (10) discussed the role of potassium in green plants and attributed its specific activity to the fact that potassium compounds present in protoplasm emit β-rays and give rise to physiological oxidation processes, thus promoting the generation of energy in the living cell. He concluded that it must necessarily follow that potassium is concerned with the building up of protein bodies in plant cells.

All of the authors cited so far agree that potassium promotes the production of higher nitrogen compounds in green plants, but Janssen and Bartholomew (9), working with cowpeas and sugar beets, found that potassium-deficient plants contained higher percentages of total nitrogen than did the check

plants. They also found that the percentage of protein nitrogen was higher in the deficient plants. The same authors (1) found that very low concentrations of potassium maintained in the culture solution sufficed for the needs of the plants, and that the potassium absorbed during the early stages of growth could be translocated and re-utilized.

There is not, as far as the writer knows, any record of culture studies carried out for the specific purpose of determining the effect of marked potassium deficiency on the quality of the grain produced by cereals. Gericke (6) has shown that the absorption of potassium nitrate by wheat plants after the time of heading is beneficial to the quality of the grain. There is evidence from various field experiments, such as that carried out by Scharnagel (15), that fertilizing wheat with potassium salts improves the quality, although, in some instances at least, it may actually lower the nitrogen content of the grain.

It seems probable that the various effects of limited potassium supply as noted in the preceding paragraphs would have an influence on the quality of wheat produced in water cultures. The present study deals with preliminary experiments on the effect of limited potassium supply on the composition and quality of wheat.

Experimental

Production of Material

The material used in this study was Marquis wheat grown in culture solutions during the spring and summer of 1933. Five lots of plants were produced, the equipment and methods described in an earlier paper (11) being used. The culture solutions supplied to these lots during the various stages of development are recorded in Table I, and the chemical composition of each culture solution in Table II.

TABLE I

Solutions supplied to the various cultures during three periods of growth

Culture No.	First month	To heading	To maturity	Abbreviated description
1	Complete	Complete	Complete	Complete
2	Complete	Complete	Tap water	Complete: water
3	Low K, with Na	Low K, with Na	Tap water	Low K, Na: water
4	Low K, with Ca	Low K, with Ca	Tap water	Low K, Ca: water
5	Complete	K-free with Ca	Tap water	Complete: K-free: water

At the end of the first month the tanks were all drained and refilled with the same solutions except that a potassium-free solution was supplied to culture 5. The small amount of potassium supplied to the low potassium cultures 3 and 4 during the first month was continued during the second.

The total amount of potassium supplied to these cultures over the whole period of development was 11.7 mgm. in the form of 0.30 cc. of molar potassium dihydrogen phosphate per litre of solution.

Ferric tartrate was added to each culture every third day for the first month, and then every second day until vegetative growth ceased.

TABLE II

CHEMICAL COMPOSITION OF CULTURE SOLUTIONS EXPRESSED AS CC. OF MOLAR SALT SOLUTION PER LITRE OF CULTURE SOLUTION

Solution	KNO_3	$Ca(NO_3)_2$	$NaNO_3$	KH_2PO_4	$Ca(H_2PO_4)_2$	NaH_2PO_4	$MgSO_4$	Ferric tartrate
Complete	5	5	—	1	—	—	2	1
Low K with Na	—	5	5	0.15*	—	0.85	2	1
Low K with Ca	—	7.5	—	0.15*	0.85**	—	2	1
K-free with Ca	—	7.5	—	—	0.85**	—	2	1
Tap water †	—	—	—	—	—	—	—	1

* *Added as 0.05 cc. once every 10 days for 2 months.*

** *Added as 6.8 cc. of* $\frac{M}{10}$ $Ca(H_2PO_4)_2$.

† *Tap water contained about 200 p.p.m. of salts but no N or K.*

When the plants were mature, the samples were collected and threshed. The total nitrogen, ash, potassium, calcium, magnesium, phosphate and sulphate contents of straw (including chaff) and grain were determined. The nitrogen compounds of the grain were studied in some detail.

METHODS OF ANALYSIS

Total nitrogen and nitrogen fractions. The methods used for determining total nitrogen and the various nitrogen fractions have been discussed in an earlier paper (11). In addition, however, arginine nitrogen in the gluten hydrolysate was determined by the procedure outlined by Plimmer and Rosedale (14).

Ash. Approximately two-gram samples of ground straw and five-gram samples of grain were ashed in a muffle furnace at 570° C. for six hours. The ash samples were taken up in dilute hydrochloric acid, and the solutions made up to 100 cc. Aliquots were used in the determination of the mineral elements.

Potassium. Potassium was determined on 5 cc. aliquots of the ash solution using the colorimetric method described by Morris and Gerdel (12).

Calcium. Calcium in 40 cc. aliquots of the ash solution was precipitated as calcium oxalate and the oxalate titrated with $N/20$ potassium permanganate.

Magnesium. Magnesium in the filtrate from the calcium determination was precipitated as magnesium ammonium phosphate, taken up with $N/14$ sulphuric acid, and the excess acid titrated with $N/14$ sodium hydroxide.

Phosphate. Phosphate was determined on 2-cc. aliquots of the ash solution using the colorimetric method as described by Truog and Meyer (16).

Sulphate. Sulphate in 50-cc. aliquots was precipitated with barium chloride, ignited and weighed as barium sulphate.

Results

The results are summarized in Tables III to XI. The discussion of the tabulated data is largely restricted to the differences resulting from potassium nutrition.

Grade and Weight of Grain

The grade, weights per bushel and weight per 1000 kernels of each of the five samples are presented in Table III. The grain from complete culture plants was de-graded because the kernels were somewhat shrivelled, although they were hard and vitreous. The cause of this shrivelling has been discussed in an earlier paper (11). It was concluded that the synthesis of organic nitrogen compounds from nitrates, which continued for some time after vegetative growth and the production of carbohydrates had ceased, used considerable quantities of soluble carbohydrates which would otherwise have been translocated to the grain as such. The removal of these carbohydrates in the vegetative parts of the plant resulted in the incomplete filling of the developing kernels, and the consequent shrivelled appearance. None of the results in the present study have given any indication that these conclusions need be altered. The low potassium, calcium: water, and complete: potassium-free: water samples were de-graded because they contained some starchy kernels.

TABLE III

Weight and grade of mature kernels

Culture No.	Description of culture	Time to maturity,* days	Grade	Weight per bushel, lb.	Weight per 1000 kernels, gm.	Grain as % of total weight of crop
1	Complete	113	2 Northern	63	33.51	33.8
2	Complete: water	106	1 Hard	65	28.64	37.0
3	Low K, Na: water	106	1 Hard	63	22.43	36.6
4	Low K, Ca: water	106	2 Northern	62.5	26.09	38.7
5	Complete: K-free: water	106	1 Northern	63.5	26.32	38.9

* *Time taken from first soaking of seeds.*

Table III shows that the weight per bushel was greatest in the grain from No. 2 culture, with probably insignificant differences among the weights of the grain from other cultures. It is obvious that weight per bushel is not related to weight per 1000 kernels, nor is weight per 1000 kernels related to grade. All the samples produced under conditions of limited potassium supply were lower in weight per 1000 kernels than were the others. The very

low weight of the sample from No. 3 is impossible to explain, unless it was the result of substituting sodium for potassium. As far as the writer knows, there is no reason to suppose that this should make such a great difference, but the work is being extended in the hope of determining more definitely the effect on weight of substituting sodium and calcium for potassium.

The differences in weights of grain as percentages of the total weight are probably not significant except in the case of the complete as compared with the other four samples. The results recorded in Tables IV and VIII show that the extra amounts of nitrogen and ash constituents absorbed by the complete-culture plants and the smaller proportion of these translocated to the kernels as compared with the plants of any of the other cultures, are alone sufficient to account for the differences in the proportion of grain to total weight. There is no evidence that there was greater vegetative growth in the complete than in other cultures.

Total Absorption of Inorganic Constituents

The total amounts of the various inorganic elements absorbed by the plants were calculated from the analyses and the proportions of straw and grain. These calculated values are presented in Table IV as grams absorbed per 100 grams of dry matter. They might have been calculated on the basis of the yields for the various cultures, but these results were considered unreliable and are not recorded.

The complete-culture plants absorbed more of each constituent determined than did any of the other plants, but this result was to be expected on account of the longer absorbing period. The absorption of nitrogen by the plants in the other four cultures was apparently not affected by potassium supply,

TABLE IV

Total absorption of nitrogen and minerals expressed as gm. per 100 gm. dry material

Culture No.	Description of culture	N, gm.	Ash, gm.	K, gm.	Ca, gm.	Mg, gm.	P_2O_5, gm.	SO_4, gm.
1	Complete	1.60*	7.95	2.16	1.40	0.27	0.71	0.54
2	Complete: water	1.07	4.38	1.69	0.73	0.20	0.35	0.16
3	Low K, Na: water	1.10	3.58	0.59	0.92	0.22	0.36	0.24
4	Low K, Ca: water	1.06	3.59	0.58	1.22	0.22	0.54	0.22
5	Complete: K-free: water	1.07	3.31	0.60	0.93	0.21	0.36	0.22

* *This figure excludes nitrate nitrogen absorbed but not reduced. The corresponding figure for total nitrogen is 2.05 gm.*

but the absorption of the ash constituents was. The total amount of ash in complete: water plants was higher than in either of those lots which had a limited potassium supply at all stages, and the ash in these in turn was higher than that in the complete: potassium-free: water plants. Since the potassium uptake of the plants in the last three cultures was the same, it

appears that the time of uptake of this element influences the total absorption of other elements. This conclusion is supported by preliminary results from a study of the distribution of the various elements during the vegetative growth of wheat plants in controlled culture solutions. The increased absorption of other elements brought about by the restriction of potassium supply at any stage of growth is insufficient to compensate for the restricted potassium uptake. Apparently complete absence of potassium from the culture solution from the time the plants are one month old onward paralyzes the absorptive power of the plant to a considerable extent. Whether this is due to the lack of the specific action of the potassium or to an upset balance is not determinable from the results so far obtained.

The absorption of potassium by the plants grown in each of the two low potassium cultures was determined by the potassium supply, and it is quite fortuitous that the plants in the complete: potassium-free: water culture should have absorbed the same amount of potassium in one month as was supplied to the other plants up to the time of heading. Owing to the unreliability of the yields it is impossible to make accurate calculations, but it is evident that practically all the potassium supplied to the two low potassium cultures was absorbed by the plants. The total potassium supplied to each culture during the whole period of development was 0.30 cc. of molar potassium per litre of solution, or 1.35 gm. per tank. The yield of dry matter from each of these tanks was approximately 300 gm. and the total yield of absorbed potassium about 1.75 gm. This is somewhat greater than the amount supplied, but probably the excess can be accounted for by the presence of potassium in the reserves of the seed, and as slight impurities in the other salts.

Considering all but the complete-culture plants, the greatest amount of calcium was absorbed by the plants grown in the low potassium, calcium: water culture, and this was accompanied by the greatest absorption of phosphate. Apparently the sodium supplied to the plants in the low potassium, sodium: water culture did not wholly compensate for the potassium even in absorption, as the calcium uptake of these plants was considerably higher than that of the plants in the complete: water culture. The amount of calcium absorbed by the plants in the complete: potassium-free: water culture was greater than that absorbed by the low potassium, calcium: water plants. Apparently the presence of small amounts of potassium increased the absorption of calcium, but the presence of large amounts depressed it.

There were no significant differences in the amounts of magnesium absorbed by any of the plants grown in tap water after heading. The magnesium level was low in all the samples.

The only significant difference in the phosphate absorptions of the last four samples was in that of the low potassium, calcium: water plants as compared with those of the other three lots. The greater absorption of phosphate has already been shown to accompany the greater absorption of calcium.

The sulphate absorption is apparently increased by limiting the potassium uptake. The amounts absorbed by all lots were small, but the difference between the absorption of the complete: water plants and that of each of the restricted potassium lots is probably significant.

Taken as a whole, the results for total absorption show that the amounts of the inorganic elements absorbed by plants growing in culture solutions are by no means wholly determined by the composition of the solutions. The complete solution in which the complete: water plants were grown until heading contained potassium and calcium in the ratio of 1 : 0.83, and the amounts of these elements absorbed were in the ratio of 1 : 0.43. The low potassium, calcium solution in which the low potassium, calcium: water plants were grown contained potassium and calcium in the ratio of 1 : 50, but the amounts of these elements absorbed were in the ratio of 1 : 2.1. It is obvious that the plants grown in the complete solution exercised a slight, and those in the low potassium, calcium solution a marked, power of selective absorption for potassium.

Composition of Straw and Grain

The results of the analyses of straw and grain are presented in Tables V to VIII. As it is impossible to discuss separately, the results recorded in each table the following paragraphs deal with all results together. In Tables IV, V and VI the figure for nitrogen in complete culture plants excludes nitrates absorbed but not reduced. The mature plants in the other four cultures contained no nitrate nitrogen as they were removed to tap water before reduction of nitrates ceased.

In general, the total absorption by the plant is reflected in the results of the straw composition. Although there were marked variations in the total absorption by the plants in the different cultures, apparently the amount of any one element absorbed did not determine the amount translocated to the kernels except in the case of nitrogen, and to a more limited extent in that of phosphorus. It is evident from the results in Tables V and VI that fairly

TABLE V

Composition of straw and grain expressed as percentages of the dry matter

Culture No.	Description of culture	N	Ash	K	Ca	Mg	P_2O_5	SO_4
Straw								
1	Complete	0.61*	10.70	2.99	2.06	0.33	0.63	0.81
2	Complete: water	0.34	5.67	2.31	1.06	0.23	0.24	0.24
3	Low K, Na: water	0.39	4.40	0.58	1.34	0.26	0.32	0.37
4	Low K, Ca: water	0.36	4.42	0.58	1.86	0.25	0.49	0.36
5	Complete: K-free: water	0.33	4.02	0.62	1.40	0.23	0.23	0.36
Grain								
1	Complete	3.52	2.58	0.54	0.13	0.16	0.84	—
2	Complete: water	2.32	2.14	0.61	0.16	0.17	0.56	—
3	Low K, Na: water	2.32	2.16	0.60	0.20	0.15	0.57	—
4	Low K, Ca: water	2.18	2.28	0.57	0.20	0.17	0.63	—
5	Complete: K-free: water	2.18	2.18	0.57	0.17	0.18	0.57	—

* *This figure excludes nitrates absorbed but not reduced. The corresponding figure for total N is 1.30%.*

constant *amounts* of potassium, calcium and magnesium are tanslocated to the kernels. It is equally evident that the amount of nitrogen in the kernels varies considerably, but from the results in Table VIII it appears that the *proportion* of the organic nitrogen translocated to the kernels is fairly constant. Thus, while the amount of potassium in the kernels from 100 gm. of dry plant material varied only from 0.18 to 0.23 gm., the proportion of the plant's total potassium translocated to the kernels varied from 8.3 to 37.9%; and, while the amount of total nitrogen in the kernels varied from 0.84 to 1.19 gm., the proportion of the plant's total nitrogen in the kernels varied only from 74.6 to 80.4%. The higher percentage of phosphorus in the kernels from complete-culture plants is probably related to the larger amount of protein in these kernels.

TABLE VI

DISTRIBUTION OF NITROGEN AND MINERALS BETWEEN STRAW AND GRAIN FROM 100 GM. OF DRY MATERIAL

Culture No.	Description of culture	Weight of straw or grain, gm.	N, gm.	Ash, gm.	K, gm.	Ca, gm.	Mg, gm.	P_2O_5, gm.	SO_4, gm.
Straw									
1	Complete	66.2	0.41*	7.08	1.98	1.36	0.22	0.42	0.54
2	Complete: water	63.0	0.21	3.59	1.46	0.67	0.14	0.15	0.16
3	Low K, Na: water	63.4	0.25	2.79	0.37	0.85	0.16	0.15	0.24
4	Low K, Ca: water	61.3	0.22	2.71	0.36	1.14	0.15	0.29	0.22
5	Complete: K-free: water	61.1	0.20	2.46	0.38	0.86	0.14	0.14	0.22
Grain									
1	Complete	33.8	1.19	0.87	0.18	0.04	0.05	0.28	—
2	Complete: water	37.0	0.86	0.79	0.23	0.06	0.06	0.21	—
3	Low K, Na: water	36.6	0.85	0.79	0.22	0.07	0.06	0.21	—
4	Low K, Ca: water	38.7	0.84	0.88	0.22	0.08	0.07	0.24	—
5	Complete: K-free: water	38.9	0.85	0.85	0.22	0.07	0.07	0.22	—

* *This figure excludes nitrate nitrogen absorbed but not reduced. The corresponding figure for total nitrogen is 0.86 gm.*

The tendency of all lots of plants to produce kernels with fairly constant mineral contents, accentuates the differences in the proportions of the elements remaining in the straw of the different samples. The complete-culture plants absorbed a little less than four times as much potassium as the plants grown in restricted potassium cultures, but the straw of the former contained more than five times as much potassium as that of the latter.

The results presented in Table VII show that the amounts of the various minerals expressed as percentages of the ash varied markedly for the whole plant and for the straw, but to a much smaller extent for the grain. The variations in the results for the whole plants are of course determined by the factors already discussed in the section on absorption, and those in the results for straw by the relation between total absorption and translocation to the

TABLE VII

MINERALS AS PERCENTAGES OF THE ASH IN WHOLE PLANT, STRAW AND GRAIN

Culture No.	Description of culture	K	Ca	Mg	P_2O_5	SO_4	Recovery of ash
Whole plant							
1	Complete	27.2	17.6	3.4	8.9	6.8	63.8
2	Complete: water	38.6	16.7	4.6	8.1	3.6	71.5
3	Low K, Na: water	16.5	25.7	6.1	10.1	6.6	64.9
4	Low K, Ca: water	16.2	34.0	6.1	15.0	6.2	77.4
5	Complete: K-free: water	18.1	28.1	6.3	10.9	6.8	70.2
Straw							
1	Complete	27.9	19.3	3.0	6.0	7.6	63.7
2	Complete: water	41.0	18.6	4.0	4.0	4.4	71.9
3	Low K, Na: water	13.8	29.1	5.6	7.7	8.8	64.0
4	Low K, Ca: water	13.2	41.5	5.5	11.5	8.3	80.0
5	Complete: K-free: water	15.4	35.2	5.8	5.7	9.0	71.1
Grain							
1	Complete	21.1	4.9	6.4	32.9	—	65.3
2	Complete: water	28.9	7.2	7.8	26.7	—	70.6
3	Low K, Na: water	27.6	9.1	6.7	26.5	—	69.9
4	Low K, Ca: water	25.2	8.7	7.3	27.7	—	68.9
5	Complete: K-free: water	26.4	7.8	8.2	26.1	—	68.5

TABLE VIII

PROPORTION OF ORGANIC NITROGEN AND MINERALS IN GRAIN

Culture No.	Description of culture	Organic N	Ash	K	Ca	Mg	P_2O_5
1	Complete	74.6	10.9	8.3	2.9	18.5	39.6
2	Complete: water	80.4	18.0	13.6	8.2	30.0	58.3
3	Low K, Na: water	77.3	22.1	37.3	7.6	27.3	59.2
4	Low K, Ca: water	79.2	24.5	37.9	6.6	31.8	45.2
5	Complete: K-free: water	79.4	25.7	36.7	7.5	33.3	61.2

kernels. The higher percentage of phosphate in the ash from complete-culture kernels, which has already been attributed to the higher protein level of these kernels as compared with that of the others, reduces the percentages of all other elements as calculated on the basis of the total ash.

NITROGEN DISTRIBUTION AND QUALITY OF GRAIN

The results presented in Tables IX to XI were obtained in studies carried out in an effort to determine the quality of the grain. The individual samples were too small to be studied by the methods usually employed in determining quality, so a detailed study of the nitrogen distribution in the grain, and of the chemical and physical nature of the gluten, was undertaken.

The results of the studies on the distribution of gluten and non-gluten nitrogen in the kernels are recorded in Table IX. These results confirm the

conclusion reached in an earlier paper (11) that the distribution of the nitrogen fractions is directly related to the total nitrogen content of the kernels. It is obvious that the gluten nitrogen is the fraction which increases with an increase in total nitrogen, as there is a spread of only 0.09% in the non-gluten nitrogen values expressed as percentages of the dry matter, as compared with a spread of 1.31% in the gluten nitrogen values on the same basis.

Although there are only two samples, there is an indication that restricting the potassium supply may result in a slight increase in the non-gluten nitrogen.

TABLE IX

DISTRIBUTION OF GLUTEN AND NON-GLUTEN NITROGEN IN GRAIN

Culture No.	Description of culture	Total N % D.M.	Gluten N		Non-gluten protein N		Non-protein N		Total non-gluten N	
			% T.N.	% D.M.	% T.N.	% D.M.	% T.N.	% D.M.	% T.N.	% D.M.
1	Complete	3.52	77.1	2.71	16.3	0.57	6.6	0.23	22.9	0.80
2	Complete: water	2.32	68.8	1.60	24.2	0.56	7.0	0.16	31.2	0.72
3	Low K, Na: water	2.32	65.0	1.51	27.4	0.63	7.6	0.18	35.0	0.81
4	Low K, Ca: water	2.18	64.3	1.40	28.4	0.62	7.3	0.16	35.7	0.78
5	Complete: K-free: water	2.18	64.2	1.40	28.8	0.63	7.0	0.15	35.8	0.78

The kernels from the low potassium, sodium: water plants contained a higher proportion of the total nitrogen and of the dry matter in the form of non-gluten nitrogen than did the kernels from the complete: water plants, although the total nitrogen contents were the same.

The results presented in Table X give some indication of the quality of the grain produced by each of the cultures. The gluten weights are largely determined by the gluten nitrogen content, but the wet glutens from samples which are lower in gluten nitrogen are slightly more than correspondingly low in weight. The dry matter contents of the various glutens show no significant variations, nor do the total nitrogen contents except in the case of the gluten from the complete: potassium-free: water as compared with the other samples. The higher percentage of nitrogen in this sample can be attributed to the coarseness of the wet gluten. It has been noted in an unpublished study that the texture of the gluten determines, to some extent at least, the ease with which the starch is washed from the dough ball in the gluten washing process. Within a particular series, the coarser gluten has the higher percentage of nitrogen due to the more complete removal of starch in a definite time.

The estimation of the quality of the various samples was necessarily based to a large extent on the characteristics of the wet gluten samples. These samples were obtained by the procedure recommended by Dill and Alsberg

(3) except that wheat meal, ground to pass a 1 mm. sieve, was used instead of flour. Admittedly this estimate is subject to all the errors of personal opinion, but the variations among the physical characters of the various glutens were so marked that there is little doubt as to the variations in quality. The dough ball made from grain grown in complete culture handled easily at all stages of the washing process, and yielded a gluten of excellent texture, elasticity and color. The dough ball made from grain grown in complete: potassium-free: water culture was very slack and hard to handle in washing, and yielded a gluten which was coarse, non-elastic and dark. The other samples were intermediate between these two in all respects, the differences being recorded in Table X.

TABLE X

WEIGHT AND NITROGEN CONTENT OF GLUTEN

Culture No.	Description of culture	Weight of gluten from 10 gm.			Nitrogen in gluten		Nature of wet gluten
		Wet, gm.	Dry, gm.	Dry as % of wet %	Wet, %	Dry, %	
1	Complete	7.10	2.39	33.7	3.82	11.2	Excellent in all respects.
2	Complete: water	4.05	1.38	34.1	3.94	11.6	Good in all respects.
3	Low K, Na: water	3.91	1.35	34.5	3.85	11.2	Fairly good; somewhat coarse.
4	Low K, Ca: water	3.57	—	—	3.93	—	Fairly good; somewhat coarse.
5	Complete: K-free: water	3.31	1.12	33.8	4.23	12.4	Poor; coarse, spongy and dark.

It is of interest to note that the complete: water culture plants did not yield as good a gluten as the complete culture plants. In the earlier paper (11) it was concluded that nitrogen supply alone did not affect the quality of the gluten. In that study all the elements of the complete culture solution except nitrogen were supplied to the limited nitrogen plants after heading, but in the present study the corresponding sample was grown in tap water. Apparently even during the later stages of development the continued

TABLE XI

NITROGEN FRACTIONS OF GLUTEN EXPRESSED AS PERCENTAGES OF GLUTEN NITROGEN

Culture No.	Description of culture	Nitrogen fractions of gluten						
		Humin	Amide	Argi-nine	Basic	Filtrate from basic	Mono-amino	Re-covery
1	Complete	1.7	22.0	10.4	16.5	61.0	49.9	101.2
2	Complete: water	1.9	21.5	9.2	14.9	63.3	48.9	101.6
3	Low K, Na: water	2.0	21.8	8.6	15.0	62.7	49.4	101.5
4	Low K, Ca: water	1.6	21.6	—	—	—	—	—
5	Complete: K-free: water	1.5	21.8	8.2	14.7	63.0	49.5	101.0

absorption of mineral elements is beneficial to the quality of the grain produced, even though no nitrogen is absorbed during these stages.

The results of the fractional analyses of gluten nitrogen (Table XI) indicate that the physical nature of the gluten is probably not determined by the chemical composition. This is in agreement with the results of many other workers. There is a possibility that the variations in the basic nitrogen fraction, and particularly arginine, may be significant, but this is impossible to determine from the results of the present limited study.

Discussion

A review of all results presented shows that the amount and nature of the minerals absorbed and the time of uptake influence the character of the grain produced by the plant. There is no doubt that the complete culture sample is the best in quality, and the differences between it and the complete: water culture sample demonstrate the value of minerals absorbed after the time of heading.

In evaluating the effect of restricted potassium supply, the complete: water sample must be considered as the control. A comparison of the low potassium, sodium: water sample and the control indicates that potassium cannot be wholly replaced by sodium either in absorption or in functions within the plant. The use of sodium is apparently somewhat beneficial as compared with complete replacement of potassium by calcium.

The poorer quality of the grain from low potassium, calcium: water and complete: potassium-free: water cultures might be the result of limited potassium supply or of too great an absorption of calcium. It is believed that a comparison of the absorption of these two samples shows that the former is the more important cause of the poor quality. The low potassium, calcium: water plants absorbed 1.22 gm. of calcium per 100 gm. of dry matter, and the complete: potassium-free: water plants absorbed 0.93 gm. Thus, although the former produced grain of decidedly better quality than the latter, the calcium uptake was about 30% greater, while the amounts of potassium and nitrogen absorbed were the same. Had the extra calcium been the cause of the poorer quality, then the quality of the grain from the complete: potassium-free: water plants would have been better than that of the grain from the low potassium, calcium: water plants.

These two samples also serve to illustrate the effect of the time of absorption of potassium. Although the total absorption was the same, the low potassium, calcium: water plants received potassium in small amounts supplied regularly from germination until the time of heading, and the other plants received it in the complete culture for one month. The difference in quality of grain from the two lots must have been the result of the time of absorption of potassium as the other factors determining development were the same.

The results of the analyses of the grain serve to emphasize the fact that it is impossible even to estimate the amounts of the various nutrients absorbed by studying the distribution of these nutrients in the grain alone. Total

nitrogen determinations of the grain give a good estimate of the nitrogen absorbed by the plant, because a fairly constant proportion of the plant's total nitrogen is translocated to the kernels. The minerals, on the other hand, are translocated to the kernels in fairly constant amounts per unit of dry weight. It therefore appears necessary, in any nutritional study, to determine the distribution of the various elements in the vegetative parts of the plant as well as in the kernels.

This investigation in somewhat modified form is being repeated and the absorption and distribution of the various elements at several stages of development are being determined. More definite measures of quality are planned, and it is hoped that the quality of the samples may be definitely and quantitatively related to the cultural treatment.

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NATURAL AND ARTIFICIAL HYBRIDIZATION OF *AVENA SATIVA* WITH *A. FATUA* AND ITS RELATION TO THE ORIGIN OF FATUOIDS[1]

By O. S. Aamodt,[2] L. P. V. Johnson[3] and J. M. Manson[4]

Abstract

The common fatuoid or false wild oat is an aberrant grain type which occurs frequently in cultivated oats. Three theories have been advanced in explanation of the origin of fatuoids, namely, chromosome aberration, gene mutation, and natural hybridization. The third theory has been much criticized in recent years. Observations in Alberta, Canada, indicated the possibility of natural hybridization between *Avena fatua* and *A. sativa* being an important factor in the origin of fatuoids. Genetic studies were made of both artificial and natural crosses between *A. fatua* and *A. sativa* which indicate the probability that the common fatuoid is a normal Mendelian segregate from the crosses in question. It is believed that more or less complete selective elimination of non-fatuoid segregates can be explained. While the authors believe that natural hybridization is the usual means by which fatuoids originate, they, nevertheless, entertain the possibilities of origin by chromosome aberration or by gene mutation.

Introduction

The origin of fatuoids, or false wild oats, has been the subject of controversy for nearly fifty years, and remains still an unsettled problem. Involved in the solution of this problem are implications both of scientific and economic importance. Accurate knowledge of the mode of origin of fatuoids might reasonably be expected to elucidate, to some extent, the general problem of variation; and also to point the way to methods for the eradication or control of fatuoids in seed stocks.

Huskins (14, 15) has classified several types of fatuoids on the basis of chromosome numbers and meiotic behavior. The present paper is concerned only with Huskins' so-called type 1, or "A" series which presumably carries the normal chromosome complement, and which will be referred to herein under the term, "fatuoid". This fatuoid may be described as follows:

The common fatuoid is an aberrant grain type occurring in cultivated oats. The homozygous form differs from the cultivated plant in three main characters of the grain, namely, in articulation, pubescence and awn development. A large horseshoe-shaped "sucker mouth" articulation is present on all grains of the spikelet; dense bushy pubescence is present on both callus and rachilla; a strong, twisted and geniculate awn is present on each grain of the spikelet. The heterozygous form is, with respect to these characters which comprise the "fatuoid complex", intermediate between the homozygous

[1] *Original manuscript received August 8, 1934.*
Contribution from the Department of Field Crops, University of Alberta, Edmonton, Canada.

[2] *Professor of Genetics and Plant Breeding, University of Alberta.*

[3] *Formerly Graduate Assistant, University of Alberta; now Graduate Student, Washington State College, Pullman, Washington, U.S.A.*

[4] *Formerly Assistant Plant Breeder, University of Alberta, now Biologist, National Research Council, Ottawa.*

form and the cultivated variety in question. It segregates in the ratio of approximately 1 homozygous fatuoid : 2 heterozygous fatuoids : 1 normal cultivated type; all segregates being, apparently, of equal vigor.

This is the fatuoid type commonly found to be of practical significance, other types being of very rare occurrence. While the occasional occurrence of fatuoids is not of great economic importance in ordinary commercial oat fields, it is, nevertheless, very troublesome in plots and fields of "Registered" and other high grades of seed oats.

In the course of investigations relating to the production of "Elite" and "Registered" seed stocks of oats at the University of Alberta in 1923-24, Huskins and Fryer (12) and Huskins (13) studied the genetic and cytological aspects of the origin of fatuoids, and came to the conclusion that "the evidence is overwhelmingly against the theory that fatuoids arise by natural crossing", and, "that some chromosome aberration, rather than a change in a single gene, is instrumental in causing their appearance." Later conclusions by Huskins, based on further genetic and cytologic investigations (14, 15, 16), are essentially the same as the above statement.

In recent years it has become increasingly evident from work at the University of Alberta with the same seed stocks as those used by Huskins and Fryer (12, 13), and from studies of commercial stocks produced by several registered seed growers, that fatuoids may originate also by means other than gene mutation or chromosomal aberration. In the seed stocks from which fatuoids have been collected, there occurred other off-types which appeared to be segregates such as one might expect from crosses between *Avena fatua* and *A. sativa*. During the winter of 1928-29 our attention was drawn by Mr. N. Lewis of the Dominion Seed Branch to a similar series of off-types occurring together with fatuoids and true wilds in registered seed stocks in the Provincial Seed-cleaning Plant at Edmonton. During the following summer (1929) numerous fields of oats were examined, and in a number of cases similar series of off-types were found, almost invariably in association with fatuoids and true wilds.

Although some of the more recent writers (14) have stated that the natural crossing theory of origin of fatuoids is untenable, the circumstantial evidence and the material at hand pointed so strongly to such an origin that further studies on the relation of crosses between *A. fatua* and *A. sativa* to the origin of fatuoids seemed to be warranted.

The studies reported herein consist of genetic analyses of the off-types found occurring naturally in registered seed stocks and fields of oats, and of an artificial cross between *A. fatua* and *A. sativa*. Since the off-types found occurring naturally appeared to be segregates from natural crosses between *A. fatua* and *A. sativa*, they have been compared with artificial hybrids from these two species with reference to morphological characteristics and segregation ratios.

Review of Literature on the Origin of Fatuoids

The earliest report of a direct study on the origin of fatuoids is that of Haussknecht, published in 1884 and cited by Zade (30). Haussknecht observed, in Thuringia, a continuous series of forms between *Avena sativa* and *A. fatua*, which he called *A. fatua* var. *transiens* or "Zwischenformen". He believed, in accord with the general belief of that time, that these forms could not have arisen from interspecific hybridization as no diminution of fertility was observed. Evidently Haussknecht believed, as did many botanists of his day, that sterility was the criterion of hybridity. The objection to the acceptance of intermediate forms because of the lack of sterility would not be valid at the present time, since it is generally accepted that hybrids between species are not necessarily sterile.

Koernicke and Werner, in a paper published in 1885, reviewed by Stanton, Coffman and Wiebe (26), reported studies on intermediate forms similar to those studied by Haussknecht which, they concluded, originated through natural interspecific hybridization. This direct contradiction of Haussknecht's conclusion marks the beginning of the controversy regarding the origin of fatuoids.

In 1900 Fischer (26) reported extensive investigations on intermediate forms. He found that these types did not possess the property of delayed germination as did *A. fatua*. He observed that intermediate forms appear "spontaneously and quickly among the weakly haired and awned, gray-kernelled winter oat", and seriously questioned their origin through natural crossing.

In the reports of Nilsson-Ehle published in 1907 and in 1911 (26) these intermediate forms were referred to for the first time as "fatuoids". The theory was advanced that the fatuoid probably originated through a loss-mutation in cultivated oats.

During the period from 1907 to 1912 Criddle (3, 26) published four papers on "white wild oats" or "false wild oats". He gave valuable information on the characteristics of fatuoids and concluded that they originated by mutation.

Zade, according to Stanton, Coffman and Wiebe (26), reported in 1912 the results of experiments with "Zwischenformen" and stated that these types originated by natural crossing between *A. fatua* and *A. sativa*. In 1914 Tschermak (26) came to the same conclusion after similar experiments.

This brief review of the earlier work is probably sufficient to recall the historical development of some of the present viewpoints. For more complete reviews, the reader is referred to Stanton, Coffman and Wiebe (26), and to Huskins and Fryer (12). In order to present more comprehensively the different hypotheses which have been advanced with reference to the origin of fatuoids, the more recent contributions will be reviewed in relation to the particular hypothesis to which each author subscribes.

Natural Crossing Hypothesis

In his book "Der Hafer", published in 1918, Zade (30) discussed his investigations with fatuoids. He stated that grain types intermediate between *A. fatua* and *A. sativa*, and of brown or yellow color, are frequently found in samples of threshed oats. In the progeny of these intermediates he observed segregation of types in the ratio, 1 *sativa* : 2 intermediate : 1 *fatua*. His description of these indicates that his *sativa*, intermediate and *fatua* types are probably the same as the types that more recent writers would call normal cultivated, heterozygous fatuoid, and homozygous fatuoid types, respectively. Zade believed that these types arose as segregates from natural crosses between *A. fatua* and *A. sativa*. The segregation from this cross, however, was not a monohybrid segregation such as was observed when the intermediates were grown. To explain this difference in breeding behavior Zade assumed that the intermediate forms were segregates, heterozygous for one factor, from a cross which had occurred several years previously. Thus, the generations from the intermediates were not F_2, F_3, etc., but F_{x2}, F_{x3}, etc. In some cases intermediate forms produced polyhybrid segregation in the F_{x2}, but, unfortunately, most of these plants were destroyed by insects.

Zade believed that further evidence in favor of the natural crossing hypothesis is provided by the fact that the intermediate types were of frequent occurrence, while mutations are known to occur very rarely. Examination of large samples of threshed seed always showed intermediate forms associated with wild oats. A positive correlation was found to exist between the number of intermediate type grains and the number of wild type grains in a given sample.

Challenged by the conclusions of Nilsson-Ehle (26), Zade refused to accept the former's statement that wild oats do not occur in the vicinity of Svalöf, Sweden (a fact submitted by Nilsson-Ehle as conclusive proof against the natural crossing hypothesis). He also pointed out that Nilsson-Ehle's seed stocks were not necessarily pure lines.

Crépin (1) studied the breeding behavior of an intermediate type plant found in a field of cultivated oats, and concluded that it was a natural hybrid of *A. fatua* and *A. sativa*. He believed that natural crossing, though of comparatively rare occurrence, accounted for the presence of intermediate types which cause farmers to say that cultivated oats degenerate into wild oats. In a later paper (2) he reported that he obtained an intermediate form similar to that described above, as the F_1 of a cross between *A. fatua* and *A. sativa*. In the F_2, segregation occurred in the ratio of 1 *fatua* : 2 intermediate : 1 *sativa*. This behavior corresponds to that observed in the generation arising from the heterozygous fatuoid.

In 1929 Tschermak (28)* published data from the study of several crosses between *A. sativa* and *A. fatua* which he believed strongly favored the natural crossing hypothesis. The *sativa* varieties that he used were Maunthers,

* *We are indebted to Dr. V. H. Florell for an English translation of this paper.*

Svalöf's Victory, Daubeney and Ligowa. The last-named variety is a side oat. He did not describe the wild parent; but the text suggests that two types were used, a non-yellow (black or brown) type with pubescent lemmas, and a yellow type with glabrous lemmas. The latter type must have closely resembled a homozygous fatuoid. The F_1 plants, being similar in all crosses, are described collectively. With respect to grain characters, the F_1 individual was more or less intermediate between the parental forms. The articulation was similar to that of the cultivated parent; awn development was fairly strong and confined almost entirely to the lower floret; pubescence of the lemma was always present to an intermediate degree; pubescence of the callus was in the form of two tufts of long and short hairs; the rachilla was, as a rule, glabrous. Summarizing, Tschermak stated that in all descriptions, except in the case of lemma pubescence, the F_1 type of grain corresponded to the accepted description of the heterozygous fatuoid. Color of lemma in the F_1 was not described; but we must infer, from the comparison with the heterozygous fatuoid, that it corresponded to that of the cultivated parent.

In the F_2 of each cross Tschermak found but three types of segregates, namely, *fatua*, intermediate and *sativa* forms, occurring in the ratio of 4 : 9 : 3. (He had evidently observed a similar segregation ratio in the generation arising from the heterozygous fatuoid.) The F_2 data of the four crosses are summarized as follows:

	fatua	intermediate	*sativa*
Combined F_2 data	158	444	132
Calculated ratio	4	9	3
Observed ratio	3.20	9	2.67

Tschermak stated that, while the F_3 population was too small to give ratios positively demonstrating a two-factor basis of inheritance, it served, nevertheless, to validate certain conclusions. Awnless, glabrous *sativa* forms bred true; *sativa* forms with a degree of awning and pubescence segregated in the ratio, 3 *sativa* : 1 *fatua*. The *fatua* forms were constant. The F_2 intermediate phenotype was shown to be expressed by four different genotypes: four-sixteenths of the F_2 intermediates produced *fatua*, intermediate and *sativa* forms in the ratio of 4 : 9 : 3; two-sixteenths produced intermediate and *sativa* forms in the ratio of 3 : 1; one-sixteenth remained constant.

A trifactorial inheritance scheme was devised. The genotype *aabbCC* was ascribed to the *sativa*, and *AABBCC* to the *fatua* parent. The following factorial interactions were assumed: *AA* gives *fatua* phenotype; *Aa* is hypostatic to *B*(*BB* or *Bb*); *B*(*BB* or *Bb*) with *Aa*, or alone, gives the intermediate phenotype; *C* is hypostatic to *AA*, *BB*, *Bb* and epistatic to *Aa* and gives the *sativa* phenotype.

Mutation Hypothesis

As stated by Nilsson-Ehle (21) in 1921, the mutation hypothesis assumes that there occurs in one of the gametes a mutation affecting the gene complex which governs the characters of the fatuoid complex. Such an abnormal

gamete, mating with a normal one, gives rise to a heterozygous fatuoid plant. This plant when selfed produces normal, heterozygous fatuoid, and homozygous fatuoid plants, in the ratio of 1 : 2 : 1.

Jones (19) has recently (1930) advanced a modified mutation hypothesis based on Huskins' (15) $\frac{ABC}{ABC}$ formula for normal *A. sativa*. (See chromosome aberration hypothesis below.) This hypothesis assumes that chromosome *B* carries the factors for *fatua* grain characters, and that chromosome *C* carries factors for *sativa* grain characters; the factors on *C* being epistatic to those on *B*. Mutation in *C* removes this epistasis, and thereby allows factors in *B* to express *fatua*-like grain characters.

Nishiyama (22) concluded in 1931, after extensive genetic and cytological observations, that the common fatuoid, with the normal chromosome number, originates by mutation in the *C* chromosome; and that fatuoids which show deviations from the normal chromosome number [*i.e.*, Huskins' (15) *B* and *C* series, Goulden's (10) families II and III, and Nishiyama's (22) strains H_{20}-35 and H_{58}-12] originate through chromosome aberrations. More recent studies by Nishiyama (23) have confirmed his earlier viewpoint and that of Jones (19). His recent report claims to have established a synoptic gene, or genes, which have their loci in the longer arm of the *C* chromosome (F_1). This "discovery of a chromosome or gene controlling the meiotic behavior has great significance in the cytology of hybrids, where phylogenetic relation of parents is often represented by the degree of synoptic affinity."

Chromosome Aberration Hypothesis

The chromosome aberration hypothesis, stated by Huskins (13, 14, 15) as an explanation of the origin of fatuoids, is based upon Winge's (29) explanation of the origin of speltoids in wheat, and like the latter involves a consideration of the phylogeny of the species concerned. The higher species of *Avena* and *Triticum* are believed to have evolved by multiplication of the chromosomes of more primitive species. In *Avena* the hexaploid species *A. sativa*, which contains most of our common cultivated varieties, has 21 pairs of chromosomes representing three sets of each of the seven chromosome pairs of the diploid species. The set, or di-triploid group, of chromosomes concerned with grain characters is represented in *A. sativa* by the formula $\frac{ABC}{ABC}$. The $\frac{B}{B}$ pair differs from the $\frac{C}{C}$ pair only in the factor or factors affecting articulation, pubescence, and awn development of the grain. $\frac{B}{B}$ carries factors which determine fatuoid grain characters; $\frac{C}{C}$ carries factors which determine normal grain characters; and $\frac{C}{C}$ is epistatic to $\frac{B}{B}$. Normal pairing of *A* with *A*, *B* with *B*, and *C* with *C* will maintain the equilibrium of the groups,

and true-breeding progeny will be produced. But, if faulty pairing takes place occasionally between *B* and *C*, gametes of the types *ABB* and *ACC* will result. The mating of *ABB* with the normal *ABC* gamete gives an $\frac{ABC}{ABB}$ zygote. It is assumed that this zygote, having an extra "dose" of fatuoid factors, develops into a heterozygous fatuoid. The resulting individual, when selfed, will segregate for normal $\frac{ABC}{ABC}$, heterozygous fatuoid $\frac{ABC}{ABB}$ and homozygous fatuoid $\frac{ABB}{ABB}$ types in the ratio of 1 : 2 : 1, respectively.

Huskins (15) describes three types of fatuoids: in A series the heterozygous type has 42 chromosomes and segregates in the ratio of 1 : 2 : 1 for normals, heterozygous fatuoids and homozygous fatuoids, all segregates being of normal 42-chromosome complement; in B series the heterozygote segregates for normals, heterozygotes and fatuoids having 42, 41 and 40 chromosomes, respectively; in C series the heterozygote segregates for normals, heterozygotes and fatuoids having 42, 43 and 44 chromosomes, respectively.

For more complete reviews of the recent literature bearing directly on the various hypotheses, the reader is referred to Jones (19) and Nishiyama (22).

Materials

Two distinct lots of material were studied. One lot consisted of F_1, F_2 and F_3 plants from an artificial cross between *A. fatua* and *A. sativa*. The other lot consisted of aberrant grain types found in threshed seed stocks and off-type plants selected in the field. The progenies of the latter group were studied in the same manner as those of the artificial crosses.

The artificial crosses were made in 1929. Reciprocal crossing was performed, but success was attained only where *A. fatua* was the pistillate parent. The failure to obtain seed in the reciprocal cross does not bear any special significance, since only a small number of cross fertilizations were attempted. Johnson (18) when continuing these studies found no difficulty in obtaining reciprocal crosses. Surface (27) found that there were no differences in either the F_1 or F_2 from reciprocal crosses between wild and Kherson oats.

The *A. fatua* parents used in these crosses were stray plants of the common wild oat, which grew adjacent to cultivated oat varieties on the plots at the University of Alberta. Seeds of each of these plants were collected and grown separately in parental check plots for three years. The type appeared to be uniform and free from genetic variation. The grain of *A. fatua* is characterized by an oblique, "suckermouth" articulation, heavy pubescence on rachilla, callus and lemma, dark brown or black lemma color, and strong geniculate awns on all grains of the spikelet. (Plate 1, *A*.) Other characteristics which distinguish this wild form are its delayed germination, when matured under certain environmental conditions, its early maturity, the ease with which it shatters at maturity and the fine drooping branches of its panicle.

The *A. sativa* parent, Selection No. 76, was sent to the University of Alberta from England in 1927 by Dr. C. L. Huskins, who had previously obtained it from Professor Shegalov of Russia. This variety came to us with the distinction of having never produced awned grains nor given rise to fatuoids. It has been tested over a period of five years at the University of Alberta in one-tenth acre plots, and in several hundreds of check rows; and still retains that distinction. It has also been tested by T. R. Stanton* at Aberdeen, Idaho, for a more limited period, with similar results. Selection No. 76 is an early maturing oat of good agronomic quality and of fair yielding capacity. The plant is short, with a relatively small, medium spreading panicle; the lemma is white and glabrous; the rachilla ranges from glabrousness to a very sparse, short pubescence; the callus exhibits sparse, short to medium pubescence; the articulation is small and not oblique (Plate 1, *B*).

The natural aberrant material was obtained from elite stock of Victory maintained at the University of Alberta, and from elite stock of Banner maintained by Mr. Nels Linden at Wetaskiwin, Alberta. Two generations of the former and one of the latter material have been grown and analyzed. Other reference to this material has been made in the introduction.

Methods

The crosses were made in the summer of 1929 and four F_1 plants were grown in the greenhouse during the winter of 1929-30.

The F_2 consisting of 69 plants was grown as a separate population. Parental check plots were grown adjacent to the hybrid plots in order to provide comparable material for study.

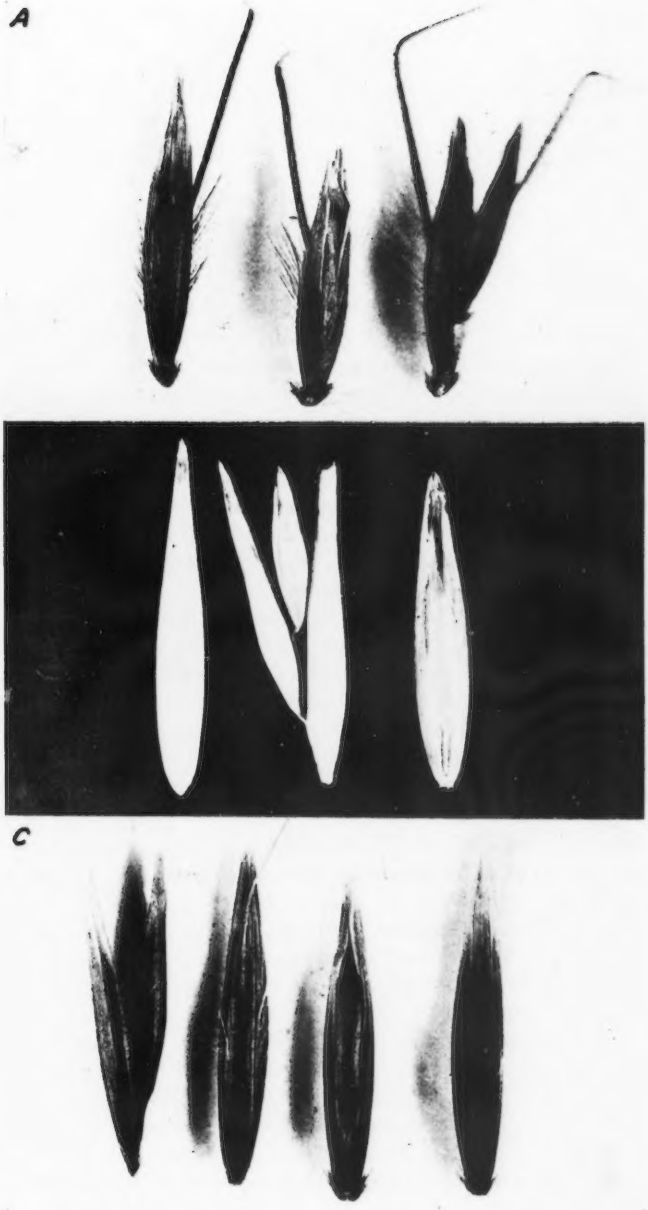
Each individual F_2 plant was thoroughly examined and classified for all the major segregating characters, especially those concerned with the fatuoid complex.

The four F_2 populations showed the same general type of segregation as far as could be expected with the limited numbers, and consequently were treated as one group in the final analysis. In all there were 69 F_2 plants. A detailed description of each plant is presented in Table X.

The F_3 was grown in the greenhouse during the summer of 1931. Thirty seeds from each F_2 plant were sown in sterilized soil in wooden flats, together with seed of both the cultivated and wild parents. The objective of this plan, in addition to providing comparative material in the morphological studies, was to determine, if possible, whether delayed germination was associated with any of the segregating characters, especially those of the fatuoid complex. Seedling emergence notes were made on each hybrid and parental plot. The number of plants in some of the F_3 lines was too small to be significant, but they were sufficiently indicative to aid in determining the genotype of the individual F_2 plants and the validity of the hypothesis used to explain the genetic ratios obtained in the study reported herein.

**From correspondence with Mr. T. R. Stanton.*

PLATE I



A. *Wild oats (A. fatua) used as a parent in crosses with Selection No. 76.* B. *Selection No. 76 (A. sativa) used as a parent in crosses with wild oats.* C. *F_1 from the cross between Selection No. 76 and wild oats.*

In all generations each plant was harvested separately, and retained intact in an individual envelope. When the seeds were removed from F_2 plants for planting, a reserve supply of seed from each plant was retained. These reserves have, by providing a means for the re-examination of the progenitors, proved to be very valuable in the analysis of F_3 families. Many difficulties encountered in the original analysis of the F_2 were cleared up when these reserves were re-examined in the light of their breeding behavior in the F_3. The same general method was followed in handling the natural aberrant material.

Experimental Results

The Artificial Cross *A. fatua* × *A. sativa*

The four F_1 plants showed a marked uniformity for all readily observable characters, the expressions of which were more or less intermediate between those of the parents. Conditions for growth were not very favorable in the greenhouse, consequently only a small number of seeds was produced by the F_1 plants. No sterile spikelets were noted. The black lemma color of the wild parent tended to be dominant, the F_1 expression being brown to dark brown. Awn development ranged from weak to strong on the primary grain, but in no case extended to the secondary grain. Rachilla pubescence varied from complete absence to an intermediate condition. Callus pubescence was expressed as sparse to medium dense tufts of short to medium-long hairs on the primary grain, but was not expressed on the secondary grain. Lemma pubescence was present on the primary grain as sparsely scattered hairs of short to medium length, but did not occur on the secondary grain. The articulation, while tending to resemble the cultivated rather than the wild type, was intermediate in many respects; it did not possess the "sucker mouth" nor the easy-shattering character of the wild oat, yet it had a far larger abscission surface and a looser connection than the cultivated oat; the plane of its position was not at a right angle to the long axis of the grain as in *A. sativa*, but tended to be oblique as in *A. fatua* (Plate 1, *C*).

In the first segregating generation (F_2) types were produced exhibiting practically all combinations between the parents in lemma color, awn development and pubescence. In the case of articulation, however, there were produced three more or less definite types, *fatua*, intermediate and *sativa*. The *fatua* or "suckermouth" type was always unmistakable. The difference between intermediate and *sativa* types was mainly a matter of size, consequently distinction between them was not always clear. F_3 breeding behavior, however, served definitely to establish these types. Since the primary objective of this investigation was to study the mode of inheritance of the fatuoid complex, only a brief statement will be made of the mode of inheritance of other characters that segregated and were studied. It can be stated that the genetic ratios obtained for each character when studied independently conforms, in general, with the results reported by other investigators; consequently it seems unnecessary to burden the reader with a detailed literature review.

Lemma Color

The grain of the F_1 plants was reddish brown to reddish black. There was considerable variation, but in no case did the color appear to be as dark as that of the wild parent.

The grain of the F_2 plants was placed in three classes, namely, black, gray and white. Di-hybrid ratios were obtained, and the numbers of plants of the respective colors agreed closely with expectation. The data obtained are compared with the theoretical expected in Table I.

TABLE I

SEGREGATION FOR COLOR IN F_2 OF A CROSS BETWEEN *A. sativa* AND *A. fatua*, AND COMPARISON WITH A THEORETICAL 12 : 3 : 1 RATIO BY THE χ^2 METHOD

Class	F_2 frequencies		O−C	$(O-C)^2$	$\frac{(O-C)^2}{C}$
	Observed	Calculated			
Black	54	51.7	2.3	5.29	.102
Gray	11	13.0	2.0	4.00	.308
White	4	4.3	0.3	.09	.021
Total	69	69.0			$\chi^2 = .431$

$P = < 0.61$

That the actual ratio obtained is a good fit to the theoretical expected is indicated by the P value of .61. The *fatua* parent evidently contained a pair of dominant factors for black (*BB*), and a pair of factors for gray (*GG*) which are hypostatic to the factors for black. The white (*bbgg*) segregate in the F_2 is recessive to both black and gray.

The breeding behavior of the F_2 plants in the F_3 was in accordance with the theoretical expectation, except for the occurrence of too many white segregates and too few of the gray segregates. Between these two colors there were several shades showing all gradations and making definite classification difficult. Other workers have also experienced difficulty in distinguishing between these two colors in classifying oat grains. Some plants which showed no trace of the gray color on the dorsal side, show a distinct color on the ventral (palea) side. Such plants also showed a darker gray on the upper grains of the spikelet than on the lower grains. These plants were usually found to be heterozygous. The homozygous grays had a more distinct color. One of the gray *fatua* segregates, which appeared homozygous for gray in the F_3 under field conditions, produced both white and gray types when grown in the greenhouse. In view of these facts it appeared safe to conclude that it was impracticable to distinguish between white and gray grain color. This was especially true in the case of the heterozygous gray types and in such cases it was necessary to group the data from gray and white grains.

Rachilla Pubescence

In the F_1 the rachilla of the lower grain was practically smooth with only an occasional short hair on some of the grains. Rachilla pubescence of the F_2 plants was divided into three classes: long and dense, as in the *fatua* parent; short and sparse; and absent, as in the *sativa* parent. Since the heterozygote varied from smooth to a condition of only a few short sparse hairs, as was indicated in the F_1, it was difficult to separate the intermediate from the *sativa* type. When these two groups were combined, a simple monohybrid ratio was obtained. The data obtained are compared with the theoretical expected in Table II.

TABLE II

SEGREGATION FOR RACHILLA PUBESCENCE IN F_2 OF A CROSS BETWEEN *A. sativa* AND *A. fatua*, AND COMPARISON WITH A THEORETICAL 3 : 1 RATIO

Class	F_2 frequencies		Dev.	P.E.	D/P.E.	Odds
	Observed	Calculated				
Smooth	53	51.75	1.25	2.43	0.514	<1 : 1
Hairy	16	17.25				
Total	69	69.00				

That the actual ratio obtained is a good fit to the theoretical expected, is indicated by odds of less than 1 : 1. The simple ratio indicates the presence of a single factor pair governing rachilla pubescence, with smooth rachilla dominant.

Callus Pubescence

In the F_1 the callus had medium-dense tufts of short to medium-long hairs on the primary grain, and none on the secondary grain. In the F_2 the plants were placed in three classes of callus pubescence, namely: long, dense, tufted pubescence as in the *fatua* parent; intermediate pubescence as in the F_1; and absence of pubescence as in the *sativa* parent. The segregation in the F_2 is that of a simple monohybrid. The data obtained are compared with the theoretical expected in Table III.

TABLE III

SEGREGATION FOR CALLUS PUBESCENCE IN F_2 OF A CROSS BETWEEN *A. sativa* AND *A. fatua*, AND COMPARISON WITH A THEORETICAL 1 : 2 : 1 RATIO BY THE χ^2 METHOD

Class	F_2 frequencies		O−C	$(O-C)^2$	$\frac{(O-C)^2}{C}$
	Observed	Calculated			
Hairy	20	17.25	2.75	7.56	.438
Intermediate	32	34.50	2.50	6.25	.180
Smooth	17	17.25	.25	.06	.004
Total	69	69.00			$\chi^2 = 0.622$

P = <0.61

That the actual ratio obtained is a good fit to the theoretical expected ratio is indicated by the high P value of .61. The *fatua* parent contains a factor for callus pubescence which is intermediate in the heterozygote and practically dominant to the factor for absence of callus hairs in the *sativa* parent.

Lemma Pubescence

In the F_1, lemma pubescence was expressed as an intermediate condition between that of the two parents. Sparsely scattered hairs of short to medium length were present on the primary grain, but absent from the secondary grain. In the F_2, the plants were placed originally in classes similar to those of the parents and in two intermediate classes, one with sparse medium-length hairs and the other with sparse short hairs. There was, however, some difficulty in differentiating between the intermediate types and the fully pubescent types in some cases, so they were all grouped in the pubescent class. The segregation in the F_2 is that of a simple monohybrid. The data obtained are compared with the theoretical expected in Table IV.

TABLE IV

SEGREGATION FOR LEMMA PUBESCENCE IN THE F_2 OF A CROSS BETWEEN *A. sativa* AND *A. fatua*, AND COMPARISON WITH A THEORETICAL 3 : 1 RATIO

Class	F_2 frequencies		Dev.	P.E.	D/P.E.	Odds
	Observed	Calculated				
Hairy	50	51.75	1.75	2.43	0.720	<1 : 1
Smooth	19	17.25				
Total	69	69.00				

That the actual ratio obtained is a good fit to the theoretical expected is indicated by the odds of less than 1 : 1. The *fatua* parent contains a factor for lemma pubescence, which is intermediate in the heterozygote and partially dominant to the factor for absence of lemma pubescence in the *sativa* parent.

Awn Development

The primary grains of the F_1 plants had weak to strong awns, but the secondary grains were completely awnless. In the F_2 the plants were classified according to whether the kernels were free of awns like the *sativa* parent, intermediate as in F_1, or strong and geniculate as in the *fatua* parent. The segregation in the F_2 seemed to be that of a simple monohybrid. The data obtained are compared with the theoretical expected in Table V.

The ratio obtained for awn development is a good fit to the theoretical expected, as is shown by the high P value of .6065. The *fatua* parent contains a factor, or factors, for awn development which is intermediate in the heterozygote.

TABLE V

SEGREGATION FOR AWNS IN F_2 OF A CROSS BETWEEN *A. sativa* AND *A. fatua*, AND COMPARISON WITH A THEORETICAL 1 : 2 : 1 RATIO BY THE χ^2 METHOD

Class	F_2 frequencies		O−C	$(O-C)^2$	$\frac{(O-C)^2}{C}$
	Observed	Calculated			
Awnless	16	17.25	1.25	1.56	0.090
Intermediate	37	34.50	2.50	6.25	0.181
Awned	16	17.25	1.25	1.56	0.090
Total	69	69.00			$\chi^2 = 0.361$

P = <0.61

Articulation

The basal articulation of the grains on the F_1 plants was intermediate and tended to resemble the *sativa* rather than the *fatua* type. It approached the cultivated parent also in resistance to shattering. In the F_2, the plants were classified in three articulation groups, namely, *sativa*, intermediate, and *fatua*, according to whether the articulation of the kernels was similar to that of the parents, or intermediate. On this basis of classification the segregation in the F_2 failed to show a close fit to a theoretical 1 : 2 : 1 ratio. The data obtained are given in Table VI.

TABLE VI

SEGREGATION FOR BASAL ARTICULATION OF THE KERNELS IN THE F_2 FROM A CROSS BETWEEN *A. sativa* AND *A. fatua*, AND COMPARISON WITH A THEORETICAL 1 : 2 : 1 RATIO BY THE χ^2 METHOD

Class	F_2 frequencies		O−C	$(O-C)^2$	$\frac{(O-C)^2}{C}$
	Observed	Calculated			
sativa	25	17.25	7.75	60.06	3.482
Intermediate	28	34.50	6.50	42.25	1.225
fatua	16	17.25	1.25	1.56	0.090
Total	69	69.00			$\chi^2 = 4.797$

P = 0.0929

Delayed Germination

The results obtained from the study of delayed germination in the F_3 indicated that, after a period of approximately nine months of after-ripening, there was only one hybrid line out of 69 that appeared to show a distinctly low germinability. The inheritance of delayed germination in hybrids from *A. fatua* and *A. sativa* is being studied further by Johnson (18).

Linkages

One of the most striking features of the segregation in the F_2 and F_3 was the close linkage existing between articulation, awn development, rachilla

pubescence and callus pubescence—the characters of the fatuoid complex. Data compiled from independent genetic studies of each of these characters do not serve to show the relationships in the inheritance of these characters. An examination of the detailed data given in Table X, however, will serve to demonstrate this fact.

Among the *sativa* segregates, the absence of pubescence and of awn development, the *sativa* parental condition, greatly predominated; but there were a number of instances where these segregates developed callus pubescence or awns or both, usually to only a slight or moderate degree. It is not believed that this apparent break-up of the linkage relationship is caused by crossing over, for in no case were *fatua* and *sativa* expressions associated. It seems probable that relatively weak genes, in addition to and independent of the factors of the linkage group, give rise to these expressions. It is also possible that certain of these types may be due to extreme environmental influences.

Linkage between articulation, awn development and pubescence of rachilla and callus in crosses between *A. fatua* and *A. sativa* has been reported by a number of investigators. The results of Surface (27) and Fedorova (6) agree in general with the present data. The reader is referred to Florell (7) for a review of the literature on the genetics of floret articulation, and to Johnson (17) for a review of the literature on the genetics of awn development and of rachilla pubescence.

Lemma color was inherited independently of the characters, awn development, articulation, rachilla pubescence and callus pubescence. The literature on the genetics of lemma color has recently been reviewed by Johnson (17).

It was found that lemma pubescence was never expressed on white grains, and that in all but four F_2 plants (See Table X, Plants 50-3, 8, 12 and 51-11, all black *sativa*, *Bw*) it was expressed on black grains. All four of these plants produced some progeny with lemma pubescence. Obviously either lemma pubescence is linked with black lemma color, or it is inhibited by the factors for white lemma color. Surface (27) and Florell (7) observed a very small proportion of black grains with glabrous lemmas. Surface also observed a few non-black grains with pubescent lemmas. Both of these workers assumed that a condition of almost complete linkage existed between the factor for black lemma color and the factor for lemma pubescence. Love and Craig (20) found complete correlation between black color and pubescence, and between yellow color and glabrousness of the lemma. They concluded that the factor for yellow color inhibited the expression of the factor for lemma pubescence. Philp (24) obtained results in crosses between *A. fatua* and *A. sativa* which indicated the presence of two factors for lemma pubescence, one linked with, and one independent of, the factor for black lemma. The factors for white lemma (the double recessive) inhibited lemma pubescence.

An inspection of the data in Table X will provide more detailed information on the characteristics of the individual F_2 plants and their breeding behavior in the F_3.

Factorial Statement of Inheritance of Differential Characters

After each character had been studied for its individual mode of inheritance, and for its genetic relationship to the other characters, the task of devising a single scheme of inheritance for the entire group of characters was undertaken. The preliminary studies on individual characters disclosed the fact that close linkage existed between the characters,—articulation, awn development, rachilla pubescence and callus pubescence. As each of these characters was essentially monogenic in inheritance, it is assumed that in this material the linkage group is controlled by a single complex gene, or a closely linked group of genes. Specimens have been observed in the material with a complexity of characters, which indicate that the gene complex is not always transmitted as a unit. Jones (19) points out "that mutations have occurred in the C chromosome showing different degrees of complexity: fatuoids, sub-fatuoids, semi-fatuoids and mere strongly awned types, indicating that the genes mutate independently". The preliminary studies disclosed the fact that lemma color was controlled by two factors which were completely independent of the gene complex. The factor for lemma pubescence appeared to be linked with the factor for black lemma color. Considering these facts together it may be assumed that the inheritance of all characters in question is determined, essentially, by three factors,—the gene complex plus the two factors for color.

The probable presence of factors for awn development and callus pubescence, in addition to the gene complex, has been disregarded for two main reasons: first, because the expressions of these genes were in all cases relatively weak and of little significance to the objective of the investigation; second, it is convenient and desirable to explain the mode of inheritance and character relationships as simply as its essential nature will permit.

On the basis of the factorial interactions several phenotypes are to be expected. In the first place, the gene complex will determine the general types, *fatua*, intermediate, and *sativa*, which are based primarily on type of articulation. Secondly, the independent factors for lemma color will produce black, gray or white forms in each of these general types. Thus, the following phenotypes may be expected: black, gray, and white, *fatua* types; black, gray, and white, intermediate types; black, gray, and white, *sativa* types.

Based on observed data, the following factorial interactions and representations are assumed:

B—represents an epistatic factor for black lemma color.

G—represents a factor for gray lemma color; it is epistatic to *b* or *g* or both, but hypostatic to *B*.

W—represents a gene complex controlling awn development, articulation and rachilla and callus pubescence; *WW* produces *fatua*, *Ww* intermediate, and *ww sativa* types.

The following symbols are given to the various expected phenotypes:

BW, black *fatua*	BI, black intermediate	Bw, black *sativa*
GW, gray *fatua*	GI, gray intermediate	Gw, gray *sativa*
bW, white *fatua*	bI, white intermediate	bw, white *sativa*

A hypothetical inheritance scheme may now be stated as follows:

P_1 *BBWWGG* × *bbwwgg*
(BW) (bw)

F_1 *BbWwGg*
(BI)

F_2		Genotype	Phenotype	F_3 behavior
1	1	*BBGGWW*	BW	Homozygous BW
2	2	*BbGGWW*	BW	3BW : 1GW
27	2	*BBGGWw*	BI	1BW : 2BI : 1Bw
	2	*BBGgWW*	BW	Homozygous BW
	4	*BbGGWw*	BI	3BW : 6BI : 3Bw : 1GW : 2GI : 1Gw
	4	*BBGgWw*	BI	1BW : 2BI : 1Bw
	4	*BbGgWW*	BW	12BW : 3GW : 1bW
	8	*BbGgWw*	BI	F_2 segregation repeated 12BW : 24BI : 12Bw : 3GW : 6GI : 3Gw : 1bW : 2bI : 1bw
1	1	*BBggWW*	BW	Homozygous BW
9	2	*BbggWW*	BW	3BW : 1bW
	2	*BBggWw*	BI	1BW : 2BI : 1Bw
	4	*BbggWw*	BI	3BW : 6BI : 3Bw : 1bW : 2bI : 1bw
1	1	*BBGGww*	Bw	Homozygous Bw
9	2	*BbGGww*	Bw	3Bw : 1Gw
	2	*BBGgww*	Bw	Homozygous Bw
	4	*BbGgww*	Bw	12Bw : 3Gw : 1bw
1	1	*bbGGWW*	GW	Homozygous GW
9	2	*bbGGWw*	GI	1GW : 2GI : 1Gw
	2	*bbGgWW*	GW	3GW : 1bW
	4	*bbGgWw*	GI	3GW : 6GI : 3Gw : 1bW : 2bI : 1bw
1	1	*BBggww*	Bw	Homozygous Bw
3	2	*Bbggww*	Bw	3Bw : 1bw
1	1	*bbGGww*	Gw	Homozygous Gw
3	2	*bbGgww*	Gw	3Gw : 1bw
1	1	*bbggWW*	bW	Homozygous bW
3	2	*bbggWw*	bI	1bW : 2bI : 1bw
1		*bbggww*	bw	Homozygous bw

The actual F_2 data conform fairly well with the theoretical expected calculated on the basis of the preceding hypothesis. The χ^2 test for the goodness of fit of observed and calculated ratios is given in Table VII.

The resulting P value of 0.2547 indicates fair conformity between these series of frequencies. Approximately 50% of the χ^2 value is due to the excessive number of homozygous black cultivated types (Bw) and homozygous gray types (Gw).

TABLE VII

SEGREGATION FOR LEMMA COLOR AND WILD-COMPLEX IN THE F_2 FROM A CROSS BETWEEN *A. sativa* AND *A. fatua*, AND COMPARISON WITH A THEORETICAL 12 : 24 : 12 : 3 : 6 : 3 : 1 : 2 : 1 RATIO BY THE χ^2 METHOD

Phenotype		F_2 frequencies		O−C	(O−C)²	$\frac{(O-C)^2}{C}$
		Observed	Calculated			
Black, wild	*BW	14	12.94	1.04	1.08	0.083
Black, intermediate	Bl	21	25.87	4.87	23.72	0.917
Black, cultivated	Bw	19	12.94	6.06	36.72	2.838
Gray, wild	GW	1	3.23	2.23	4.97	1.539
Gray, intermediate	Gl	4	6.47	2.47	6.10	0.943
Gray, cultivated	Gw	6	3.23	2.77	7.67	2.375
White, wild	bW	1	1.08	.08	.06	0.056
White, intermediate	bl	3	2.16	.84	.71	0.329
White, cultivated	bw	0	1.08	1.08	1.17	1.083
Total		69	69.00			$\chi^2 = 10.163$

P = 0.2547

* *See p. 716 for legend of symbols used.*

As a further check on the validity of the hypothesis, a comparison was made between theoretical and actual frequencies of occurrence of F_2 genotypes. The genotypes of F_2 individuals were, with few exceptions, determinable through observation of the individual's breeding behavior in the F_3. Certain groupings of genotypes were necessary owing to inability to determine the specific genotypes, either because of the epistasis of factor *B* in black phenotypes, or because of environmental conditions which prevented distinction between gray and white phenotypes in the F_3. The actual genotypic frequencies, determined from the F_3, are given in Table X. The fact that it was possible to determine the F_2 genotypes from F_3 data, and the fact that there is such close general correspondence between actual and expected frequencies of these genotypes, give strong support to the validity of the hypothesis.

The appearance of three heterozygous fatuoid types (bI) and one homozygous fatuoid type (bW) as Mendelian segregates from this artificial cross, is especially interesting since it furnishes definite proof that these types can originate as a result of hybridization between *A. sativa* and *A. fatua*. Their further appearance in the F_3 as Mendelian segregates from such F_2 plants as *BbWw* and *BbWW* makes the proof rather conclusive. In morphological characteristics and in breeding behavior these "synthetic" fatuoid types correspond exactly to the common fatuoid described in the introduction to this paper and illustrated in Plate 2, *C*.

Natural Hybrids

The occurrence of natural crossing between *A. sativa* and *A. fatua* has been proved beyond any doubt. Coffman and Wiebe (4) observed several instances of natural crossing between *A. fatua* and *A. sativa* at Aberdeen, Idaho. Derick

(5) found 0.1% of natural crossing between these species at Ottawa, Canada. Crépin (1) found a plant in a field of oats in France which was shown by breeding tests to have had its origin through a natural cross between *A. fatua* and *A. sativa*. Natural crossing between varieties of *A. sativa* has been shown to vary in frequency of occurrence from a small fraction of one per cent to several per cent. The literature on this subject has been reviewed at considerable length by Stanton and Coffman (25), and more recently by Harrington (11).

A study of the amount of natural crossing that may take place between *A. sativa* and *A. fatua* when grown in adjacent rows has been made by Johnson (18) as part of a study complementary to the investigation reported herein. He found 0.362% of natural hybrids in *A. fatua* when grown adjacent to Victory (*A. sativa*), and 0.056% natural hybrids in the Victory.

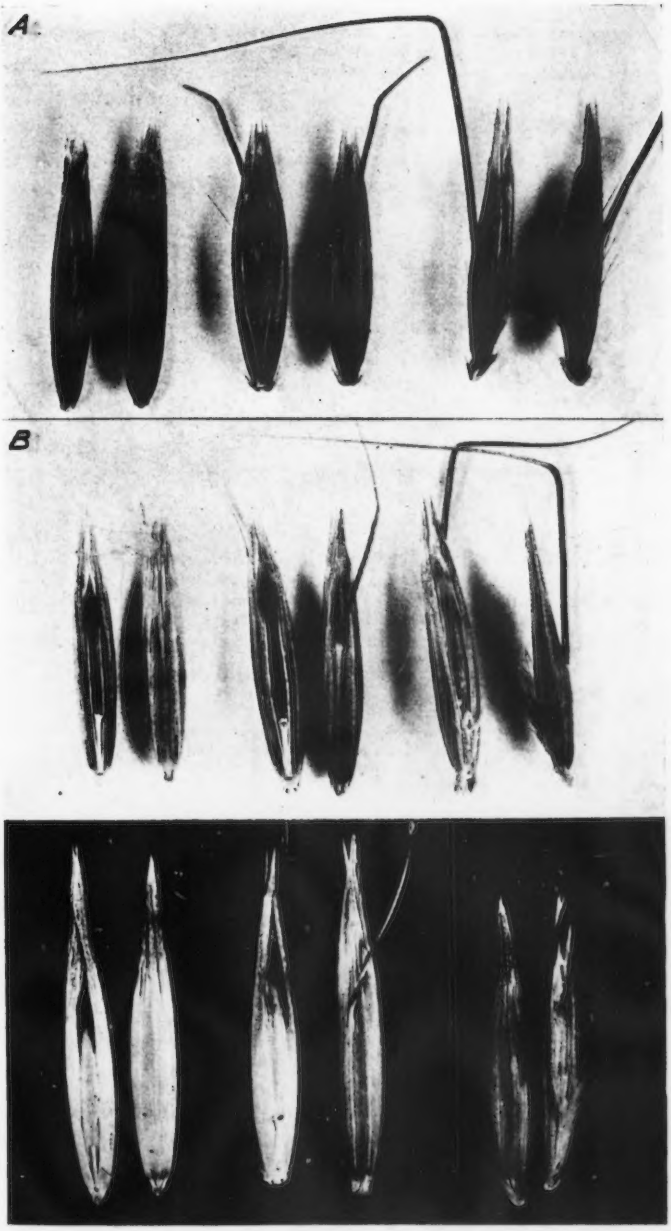
As indicated earlier in this paper, aberrant types that appeared to be segregates from natural crosses between *A. sativa* and *A. fatua* were found in seed stocks and fields of oats. Many of these were grown and studied in the same way as the segregates from the artificial crosses between these same species. Some of the natural hybrids proved to be homozygous, while others were heterozygous. All of the latter group produced segregating populations which corresponded more or less closely to certain types of segregation observed in the F_3 from the artificial cross.

Plants from 41 seeds from a black aberrant of the BI type, found in Victory oats at the University of Alberta, Edmonton, segregated in the following generation with a ratio of 32 black (B) to 9 non-black (G and b). The numbers were too small in the recessive group to expect a good ratio, and consequently they were combined. The ratio of 32 black to 9 non-black is a good fit to the theoretical expected 3 : 1 ratio with odds of less than 1 : 1 against the occurrence of a deviation as great as, or greater than this one, on the basis of random sampling. The complete data on the breeding behavior of this line are given in Table VIII, together with the theoretical expected based on the segregation of similar types in the F_3 from the artificial cross.

The test of goodness of fit by the χ^2 method shows a fair agreement between the observed and calculated frequencies, with a P value of 0.3268. Nine of the plants were typical wild oats (BW), three were typical homozygous fatuoids (bW), and four were typical heterozygous fatuoids (bI).

Thirty-nine plants were grown from a grayish aberrant of the GI type obtained from a field of Banner at Wetaskiwin, Alberta. This line segregated for gray and white and the gene complex. The data on the breeding behavior are given in Table IX, together with the theoretical expected based on the segregation of similar types in the F_3 from the artificial cross.

The test of goodness of fit by the χ^2 method shows an excellent agreement between the observed and calculated frequencies, the P value being 0.8622. Five of the plants were gray wilds (GW), two were typical homozygous fatuoids (bW), and four were typical heterozygous fatuoids (bI).



A. *A. sativa* × *A. fatua*, F_3 *black segregates. Left to right in pairs: sativa, intermediate, and fatua types.* B. *A. sativa* × *A. fatua*, F_3 *gray segregates. Left to right in pairs: sativa, intermediate, and fatua types.* C. *A. sativa* × *A. fatua*, F_2 *white segregates. Left to right in pairs: sativa, intermediate, and fatua types.*

TABLE VIII

BREEDING BEHAVIOR OF AN *A. sativa* × *A. fatua* NATURAL HYBRID AND COMPARISON OF OBSERVED AND THEORETICAL RATIOS BY THE χ^2 METHOD

Phenotype	F_2 frequencies		O−C	$(O-C)^2$	$\frac{(O-C)^2}{C}$
	Observed	Calculated			
BW	9	7.7	1.3	1.69	0.219
BI	17	15.3	1.6	2.56	0.166
Bw	6	7.7	1.7	2.89	0.375
GW and bW	2 + 3	2.6	2.4	5.76	2.215
GI and bI	0 + 4	5.1	1.1	1.21	0.237
Gw and bw	0	2.6	2.6	6.76	2.600
Total	41	41.0			$\chi^2 = 5.812$

P = 0.3268

TABLE IX

BREEDING BEHAVIOR OF AN *A. sativa* × *A. fatua* NATURAL HYBRID AND COMPARISON OF OBSERVED AND THEORETICAL RATIOS BY THE χ^2 METHOD

Phenotype	F_2 frequencies		O−C	$(O-C)^2$	$\frac{(O-C)^2}{C}$
	Observed	Calculated			
GW	5	7.31	2.31	5.34	0.731
GI	18	14.63	3.37	11.36	0.776
Gw	8	7.31	0.69	0.48	0.066
bW	2	2.44	0.44	0.19	0.078
bI	4	4.87	0.87	0.76	0.156
bw	2	2.44	0.44	0.19	0.078
Total	39	39.00			$\chi^2 = 1.885$

P = 0.8622

Many other examples could be given of aberrant types, or natural hybrids from *A. sativa* × *A. fatua* crosses, that give rise to fatuoid types which are morphologically identical to heterozygous and homozygous fatuoids. The above examples are sufficient to demonstrate that some of the aberrant types originated from natural crosses between *A. fatua* and *A. sativa*, and that certain segregates from these natural hybrids are typical fatuoids.

TABLE X

DESCRIPTION OF PARENT AND INDIVIDUAL F_2 PLANTS FROM CROSSES BETWEEN *A. fatua* AND *A. sativa*, AND THEIR BREEDING BEHAVIOR IN THE F_3*

F_2 plant No.	Description of F_2 plants								F_3 breeding behavior	
	Lemma color	Awn	Pubescence			Articulation	Phenotype	Genotype	Expected ratio	Observed data
			Rachilla	Callus	Lemma					
Wild	Black	VSt_3	VL_3	L_3	L_3	aa_3	BW	*BBGGWW*	Homozygous BW	Homozygous BW
Sel. 76	White	0	0	0	0	AA_1	bw	*bbggww*	Homozygous bw	Homozygous bw
49-1	Black	VSt_3	VL_3	L_3	L_{3+}	aa_3	BW	*BB - - WW*	Homozygous BW	27BW
2	White	I_{1-}	0	M_3	0	AA_2	bI	*bbggWw*	1bW : 2bI : 1bw	6bW : 17bI : 5bw
3	Brown to black	VSt_3	VL_2	L_3	L_3	aa_3	BW	*BbggWW*	3BW : 1bW	19BW : 6bW (1B1)†
4	Brown to black	ISt_1	Sh_1	M_{3+}	VL_3	AA_2	BI	*BB - - Ww*	1BW : 2BI : 1Bw	4BW : 15BI : 4Bw
5	Brown to black	VSt_3	VL_3	L_3	L_{3+}	aa_3	BW	*BbggWW*	3BW : 1bW	20BW : 4bW
6	Gray	VSt_3	VL_3	L_{3+}	0	aa_3	GW	*bbGgWW*	3GW : 1bW	6GW : 17bW
7	Brown to black	ISt_{1+}	0	L_3	M_1	AA_2	BI	*BbggWw*	3BW : 6BI : 3Bw : 1bW : 2bI : 1bw	3BW : 6BI : 3Bw : 1bW : 1bw
8	Brown to black	0	0	0	M_3	AA_1	Bw	*BB - - ww*	Homozygous Bw	17Bw
9	Brown to black	Wk-St_3	0	Sh_2	M_3	AA_2	BI	*BbGgWw*	F_2 ratio*	2BW : 12BI : 7Bw : 1GW : 1GI : 1Gw : 3bW : 2BI
10	Brown to black	ISt_1	Sh_{1+}	M_{3-}	L_{3+}	AA_2	BI	*BbggWw*	3BW : 6BI : 3Bw : 1bW : 2bI : 1bw	4BW : 9BI : 4Bw : 1bW : 3bI : 1bw
12	Dark brown	ISt_3	0	M_{3+}	M_{3+}	AA_2	BI	*BbggWw*	3BW : 6BI : 3Bw : 1bW : 2bI : 1bw	3BW : 12BI : 4Bw : 2bW : 2bI
13	Brown to black	ISt_{3-}	0	M_{3+}	Sh_{1+}	AA_2	BI	*BbggWw*	3BW : 6BI : 3Bw : 1bW : 2bI : 1bw	3BW : 6BI : 5Bw : 2bW : 3bI : 1bw
14	Brown to black	Wk_{1+} (long)	0	M_3	L_3	AA_2	BI	*BbGgWw*	F_2 ratio	2BW : 10BI : 6Bw : 2GI : 1bW : 1bI
15	Brown to black	ISt_{3-}	0	M_3	M_1	AA_{2-3}	BI	*BbggWw*	3BW : 6BI : 3Bw : 1bW : 2bI : 1bw	2BW : 9BI : 6Bw : 6bI : 1bw
50-1	Brown to black	VSt_3	VL_3	L_3	VL_3	aa_3	BW	*BbggWW*	3BW : 1bW	14BW : 8bW
2	Brown to black	0	0	0	Sh_{1-}	AA_1	Bw	*Bbggww*	3Bw : 1bw	15Bw : 8bw
3	Brown to black	ISt_3	0	M_{3+}	0	AA_2	BI	*BbggWw*	3BW : 6BI : 3Bw : 1bW : 2bI : 1bw	5BW : 9BI : 6Bw : 1bW : 2bI : 1bw
4	Brown to black	ISt_1	Sh_{1-}	L_{3+}	M_3	AA_2	BI	*BbggWw*	3BW : 6BI : 3Bw : 1bW : 2bI : 1bw	4BW : 6BI : 3Bw : 1bW : 2b —

* *F_2 ratio*—12BW : 24BI : 12Bw : 3GW : 6GI : 3Gw : 1bW : 2bI : 1bw.

† *Bracketed observed data are obvious mixtures or environmental variations.*

See page 723 for explanation of symbols and subscripts.

TABLE X—*Continued*

DESCRIPTION OF PARENT AND INDIVIDUAL F_2 PLANTS FROM CROSSES BETWEEN *A. fatua* AND *A. sativa*, AND THEIR BREEDING BEHAVIOR IN THE F_3*

F_2 plant No.	Description of F_2 plants								F_3 breeding behavior	
	Lemma color	Awn	Pubescence			Articulation	Phenotype	Genotype	Expected ratio	Observed data
			Rachilla	Callus	Lemma					
5	Brown to black	St_3-	Sh_1-	M_1	L_3	AA_2	BI	*BbGGWw*	3BW : 6BI : 3Bw : 1GW : 2GI : 1Gw	5BW : 5BI : 1GI
6	Brown to black	Wk_1-	Sh_1-	M_2	M_{1+}	AA_{1-2}	BI	*BbggWw*	3BW : 6BI : 3Bw : 1bW : 2bI : 1bw	5BW : 8BI : 5Bw : 3bW : 1bI
7	Brown to black	VSt_3	VL_3	L_3	L_{2+}	aa_2	BW	*BbggWW*	3BW : 1bW	19BW : 4bW
8	Brown to black	0	0	0	0	AA_1	Bw	*BbGgww*	12Bw : 3Gw : 1bw	10Bw : 2Gw : 1bw
9	Brown to black	VSt_3	VL_3	L_3	L_{2+}	aa_2	BW	*BbggWW*	3BW : 1bW	20BW : 5bW
10	Brown to black	0	0	0	M_2	AA_1	Bw	*BB - - ww*	Homozygous Bw	29Bw
11	Brown to black	VSt_3	VL_3	L_3	L_{2+}	aa_2	BW	*BbggWW*	3BW : 1bW	15BW : 4bW
12	Brown to black	Wk_1	0	M_2	0	AA_{1-2}	Bw	*BbGgww*	12Bw : 3Gw : 1bw	18Bw : 4Gw : 4bw
13	Brown to black	St_3	Sh_1-	M_2	M_2	AA_{1-2}	BI	*BbGgWw*	F_2 ratio	4BW : 8BI : 4Bw : 1GI : 1Gw : 3bW : 1bI
14	Brown to black	0	0	0	M_2	AA_{1-2}	Bw	*BB - - ww*	Homozygous Bw	24Bw (1bw)
15	Gray	0	0	0	0	AA_{1-2}	Gw	*bbGgww*	3Gw : 1bw	Immature Gw and bw
16	Black	VSt_3	VL_3	L_3	L_3	aa_2	BW	*BB - - WW*	Homozygous BW	14BW (1bW : 2bw)
51-1	Black	VSt_3	VL_3	L_3	L_3	aa_2	BW	*BbggWW*	3BW : 1bW	10BW : 2bW
2	Brown to black	St_3	Sh_1	L_3-	M_1	AA_2	BI	*BbggWw*	3BW : 6BI : 3Bw : 1bW : 2bI : 1bw	5BW : 9BI : 7Bw : 2bW : 5bI : 1bw
3	Brown to black	St_3	0	0	L_3	AA_{1-2}	Bw	*BB - - ww*	Homozygous Bw	23Bw
4	Black	VSt_3	VL_3	L_3	L_3	aa_2	BW	*BB - - WW*	Homozygous BW	28BW (Bw)
5	Gray-brown	St_3	0	M_1-	0	AA_1	Gw	*bbGGww*	Homozygous Gw	25Gw
6	Brown to black	0(VSt)	0	M_1	M_1	AA_2	Bw	*Bbggww*	3Bw : 1bw	18Bw : 4bw
7	Gray-brown to black	St_3	0	Sh-M_{1+}	M_1	AA_2	BI	*BbggWw*	3BW : 6BI : 3Bw : 1bW : 2bI : 1bw :	9BW : 5BI : 4Bw : 2bW : 1bI : 2bw
8	Brown to black	St_3	0	M_2-	M_1	AA_2	BI	*BbggWw*	3BW : 6BI : 3Bw : 1bW : 2bI : 1bw	2BW : 5BI : 2Bw : 2bI : 1bw
9	Brown to black	St_3	0	M_2	L_3	AA_2	BI	*BB - - Ww*	1BW : 2BI : 1Bw	4BW : 12BI : 8Bw
10	Black	VSt_3	VL_3	L_3	L_3	aa_2	BW	*BbggWW*	3BW : 1bW	15BW : 5bW

* *F_3 ratio*—12BW : 24BI : 12Bw : 3GW : 6GI : 3Gw : 1bW : 2bI : 1bw.
Bracketed observed data are obvious mixtures or environmental variations.

TABLE X—*Continued*

DESCRIPTION OF PARENT AND INDIVIDUAL F_2 PLANTS FROM CROSSES BETWEEN *A. fatua* AND *A. sativa*, AND THEIR BREEDING BEHAVIOR IN THE F_3*

F_2 plant No.	Description of F_2 plants								F_3 breeding behavior	
	Lemma color	Awn	Pubescence			Articulation	Phenotype	Genotype	Expected ratio	Observed data
			Rachilla	Callus	Lemma					
11	Brown to black	I_1	0	0	0	AA_1	Bw	*Bbggww*	3Bw : 1bw	17Bw : 7bw
52-1	Gray	Wk_1–	0	M_1–	0	AA_1	Gw	*bbGgww*	3Gw : 1bw	7Gw : 1bw
2	Cream	I_1+	0	M_2	0	AA_{2-3}	bI	*bbggWw*	1bW : 2bI : 1bw	2bW : 10bI : 7bw
3	Brown to black	ISt_1+	0	L_2	M_3	AA_{2-3}	BI	*BB - - Ww*	1BW : 2BI : 1Bw	10BW : 15BI : 5Bw
4	Brown to dark brown	Wk_1–	0	M_2+	M_2	AA_{2-3}	BI	*BbGgWw*	F_2 ratio	7BW : 10BI : 6Bw : 1GW : 1GI : 1bW
5	Gray	I_1	0	M_3	0	AA_2	GI	*bbGgWw*	3GW : 6GI : 3Gw : 1bW : 2bI : 1bw	1GW : 9GI : 6Gw : 4bW : 4bI : 4bw
6	Cream	VSt_3	VL_3	L_3	0	aa_3	bW	*bbggWW*	Homozygous bW	26bW
7	Brown to black	0	0	0	L_3	AA_{1-2}	Bw	*BB - - ww*	Homozygous Bw	20Bw
8	Gray	St_3+	0	M_3+	0	AA_{2-3}	GI	*bbGgWw*	3GW : 6GI : 3Gw : 1bW : 2bI : 1bw	3GW : 4GI : 5Gw : 3bW : 4bI : 5bw
9	Brown to black	0	0	0	M_1	AA_1	Bw	*BB - - ww*	Homozygous Bw	19Bw
10	Brown to black	0	0	Sh_1	L_3	AA_{1-2}	Bw	*BB - - ww*	Homozygous Bw	20Bw
11	Brown to black	VSt_3	VL_3	L_3	L_3	aa_3	BW	*BbggWW*	3BW : 1bW	15BW : 5bW (1BI : 1bI)
12	Brown to black	Wk_1	0	0	M_2	AA_1	Bw	*BB - - ww*	Homozygous Bw	30Bw
13	Brown to black	VSt_3	VL_3	L_3	L_3	aa_3	BW	*BbggWW*	3BW : 1bW	12BW : 11bW
14	Brown to dark brown	ISt_3	0	M_2	Sh_1–	AA_2	BI	*BbggWw*	3BW : 6BI : 3Bw : 1bW : 2bI : 1bw	4BW : 11BI : 4Bw : 1bW : 2bI : 3bw
15	Cream	I_1	0	M-L_3-	0	AA_{2-3}	GI	*bbGgWw*	3GW : 6GI : 3Gw : 1bW : 2bI : 1bw	1GW : 7GI : 4Gw : 3bW : 4bI : 1bw
16	Brown to black	Wk_1	0	0	L_3	AA_{1-2}	Bw	*BB - - ww*	Homozygous Bw	28Bw
17	Gray	0	0	0	0	AA_1	Gw	*bbGgww*	3Gw : 1bw	10Gw : 3bw
18	Brown to black	VSt_3	VL_3	L_3	L_3	aa_3	BW	*BB - - WW*	Homozygous BW	23BW (3BI)
19	Brown to black	Wk_1–	0	Sh_1–	M_1	AA_{1-2}	Bw	*BbGGww*	3Bw : 1Gw	18Bw : 8Gw
20	Brown to black	0	0	0	M_2	AA_1	Bw	*BB - - ww*	Homozygous Bw	25Bw
21	Brown to black	0	0	0	L_2	AA_{1-2}	Bw	*BB - - ww*	Homozygous Bw	24Bw
22	Gray	Wk_1	0	M_2+	0	AA_{2-3}	Gw	*bbGgww*	3Gw : 1bw	9Gw : 15bw

* *F_3 ratio*—12BW : 24BI : 12Bw : 3GW : 6GI : 3Gw : 1bW : 2bI : 1bw.
Bracketed observed data are obvious mixtures or environmental variations.

TABLE X—*Concluded*

DESCRIPTION OF PARENT AND INDIVIDUAL F_2 PLANTS FROM CROSSES BETWEEN *A. fatua* AND *A. sativa*, AND THEIR BREEDING BEHAVIOR IN THE F_3

F_2 plant No.	Description of F_2 plants								F_3 breeding behavior	
	Lemma color	Awn	Pubescence			Articulation	Phenotype	Genotype	Expected ratio	Observed data
			Rachilla	Callus	Lemma					
23	Gray	Wk_1	0	M-L_{2+}	0	AA_2	GI	*bbGgWw*	3GW : 6GI : 3Gw : 1bW : 2bI : 1bw	1GW : 5GI : 7Gw : 1bw
24	Brown to black	I_2	0	Sh_2	Sh_1	AA_2	BI	*BbGGWw*	3BW : 6BI : 3Bw : 1GW : 2GI : 1Gw	3BW : 7BI : 3Bw : 1GW : 4GI : 4Gw (2bW)
25	Cream	Wk_{1-}	0	M-L_{2+}	0	AA_2	bI	*bbggWw*	1bW : 2bI : 1bw	3bW : 17bI : 6bw (4Bw)
26	Brown to black	0	0	Sh_{1-}	Sh_{1-}	AA_1	Bw	*BbGGww*	3Bw : 1Gw	11Bw : 5Gw
27	Cream	0	0	0	0	AA_1	Gw	*bbGGww*	Homozygous Gw	24Gw (3bw)
28	Brown to black	0	0	0	L_3	AA_{1-2}	Bw	*BB - - ww*	Homozygous Bw	21Bw

Explanation of symbols and subscripts used in describing grain characters:

Awns

VSt—*very strong*, St—*strong*, ISt—*intermediate-strong*, I—*intermediate*, Wk—*weak*, O—*absent*.

Articulation

aa—*wild type*, AA—*semi-solidified type*.

Pubescence

VL—*very long*, L—*long*, M—*medium length*, Sh—*short*, O—*absent*.

Phenotype—Lemma color

B—*black*, G—*gray*, b—*white*.

Phenotype—for expression of the fatuoid complex of characters.

W—*wild*, I—*intermediate*, w—*cultivated*.

Numerical subscripts to all symbols denote frequency, or degree, of the character expression on a scale ranging as follows: 0, 1−, 1+, 2−, 2, 2+, 3− to 3. Thus L_3 *describes the condition of many long hairs, and* S_1 *the condition of few short hairs.*

In the case of awns, the subscript 2 *indicates the presence of awns on every spikelet of the panicle; in the case of pubescence, it indicates, approximately, the maximum hairiness of the character in question to be observed in the material; in the case of articulation it indicates large size of attachment.*

Bracketed observed data are obvious mixtures or environmental variations.

General Discussion and Conclusions

It has been demonstrated that there are two grain types occurring in the segregation from *A. fatua* × *A. sativa* crosses, which are morphologically and genetically identical with heterozygous and homozygous fatuoids, respectively.

The opponents of the natural crossing hypothesis may contend that these grain types are not true segregates from the cross, but that they have originated through chromosome aberration as a result of chromosomal incompatibilities introduced by the interspecific nature of the crosses. Huskins (16) has recently stated that "the origin of fatuoids or speltoids from crosses of *A. sativa* and *T. vulgare* with other species of oats or wheat, respectively, is of course expected on the chromosome aberration explanation, especially since hybridization increases the frequency of irregular pairing. But the origin of fatuoids in the progeny of intervarietal or interspecific crosses by no means supports the earlier hypothesis of Tschermak and others, that crossing between *A. sativa* and *A. fatua* is *the* cause of their origin. It is rather an incidental issue that they sometimes arise from such crosses". Close study of the results of the present investigation will show that such a contention is untenable. The presence of a single genetic factor for articulation; the existence of a very close linkage relationship between the factor for articulation and the factors for awn development, rachilla pubescence and callus pubescence; and the presence of two factors for lemma color which are independent of the linkage group: these facts make segregation for the two fatuoid types in *A. sativa* × *A. fatua* crosses inevitable. That the mode of inheritance of each of these characters is according to Mendelian laws, when treated independently, strengthens the conclusion that the inheritance of the gene complex is also according to Mendelian laws. Huskins (14) states that "In general it seems safe to assume that all intermediate forms which on selfing give complex segregation involving a number of characters, have arisen from the crossing of *A. sativa* and *A. fatua*". We are in complete agreement with this opinion. When fatuoids appear as segregates from such hybrid forms in normal genetic ratios, there can be no doubt that they originate through the process of hybridization and recombination. The point which really needs to be explained is not the appearance of the fatuoid, but the more or less complete selective elimination of segregates other than fatuoids.

In the material investigated the fatuoid types occurred with relative rarity in the segregating population; the various black and gray types were far more numerous. How is it then that fatuoids are not found invariably in association with a host of black and gray off-types? The answer is that fatuoids have been found with considerable regularity in association with these other aberrants. This is particularly true in fields grown from seed stocks which have not undergone selection for a considerable time. Field inspectors state that these types are of common occurrence throughout the country, particularly in districts where unselected seed is used. One inspector tells of observing many bushels of oats in which segregating types were as

numerous as the *sativa* types. A small percentage of the segregates were what are commonly called fatuoids. The University of Alberta, and several private seed growers, have found it necessary from time to time to remove black and gray off-types, in addition to fatuoids, from registered seed stocks. It is undoubtedly true, however, that in well-selected seed stocks, such as are found at experiment stations and on seed farms, fatuoids are usually found unassociated with other off-types. When selection is practised in a field or plot, the black and gray segregates from a natural cross would be readily observed and removed, the homozygous fatuoid types would also be removed, but the heterozygous fatuoid type, similar in color to the cultivated parent would, in many cases, escape notice and be propagated with the selected seed stock.

Garber (8) in disagreeing with Zade's statement that the single factor difference in heterozygous false wild oats could be accounted for by the elimination of other factors in earlier generations of the cross, states that "one must account for the selective elimination of all phenotypes except the three types, cultivated, intermediate and true false wild oats". Our observations in the field, and in the seed cleaning plants, have shown that the selective elimination of all phenotypes except the three types mentioned above, is exactly what one would expect to take place following hybridization of *A. fatua* with *A. sativa* in cultivated oat fields in which selection is practised. In rogueing in the field all the segregating types except the heterozygous fatuoid and the true *sativa* type are so distinctively off-type that they are readily recognized and eliminated. The same is true in the hand picking of pure seed stocks. This opinion is substantiated further by the fact, as stated by Huskins (14), that "In nearly all recorded cases the heterozygous form appears first." And the possibility of the fatuoid type being maintained to the exclusion of other segregating types is enhanced, as suggested by Garber (8), in that, "their further dissemination is facilitated by natural crossing".

The extent to which the heterozygous fatuoid will escape detection in a given variety, probably varies directly with the degree of awn development in that variety; for under field conditions the strong development of awns is the only feature by which the heterozygous fatuoid plant can readily be distinguished from the normal plant, hence if the normal plant is also strong-awned there will be little or no distinction. This theory is borne out by the fact that fatuoids have never been known to occur in Selection 76, which is entirely awnless, while they occur quite frequently in our common, awned varieties. All that has been said leads up to Zade's statement (30) that the heterozygous fatuoids are not F_1 types directly from a natural cross, but are segregate types from a cross which occurred several years before.

The writers are convinced that fatuoids arise through natural crossing between *A. fatua* and *A. sativa*. But, while they are certain that fatuoids originate from this cross, they also admit the possibility of origin in other ways. The incompatibility of chromosomes brought together in crosses between *A. fatua* and *A. sativa* may at times produce fatuoids through chromo-

somal aberration. Jones (19) as a result of his studies on this problem, does not question the validity of the chromosome aberration hypothesis, but does maintain that it is inapplicable to Huskins "A" series showing normal segregation and chromosome number. The present results are held to refute neither the chromosomal aberration theory, nor the gene mutation theory, regarding the origin of fatuoids, but they do support the hypothesis that fatuoids may originate also as normal Mendelian segregates from crosses between *A. fatua* and *A. sativa*.

Summary

1. Numerous aberrant types of oats, including fatuoids, intermediate between cultivated (*A. sativa*) and wild (*A. fatua*) oats were found in fields of oats and in various seed stocks.

2. It has been demonstrated that many of these aberrants originated through natural hybridization between cultivated and wild oats.

3. Artificial crosses were made between cultivated oats and wild oats. An array of types was produced in the F_2 and F_3 similar to the aberrants occurring naturally in the field.

4. Genetic studies demonstrated conclusively that these intermediate types were the result of normal Mendelian segregation.

5. Fatuoids, or false wild oats, identical morphologically and genetically with the common fatuoid, appeared as normal Mendelian segregates from both the artificial and natural crosses.

6. The non-fatuoid type segregates are eliminated more or less as a result of selection.

7. These results are not held to disprove the chromosome aberration or gene mutation theories of origin; but they are believed to establish definitely the fact that fatuoids may frequently originate through natural hybridization between *A. sativa* and *A. fatua*.

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A MATHEMATICAL THEORY OF THE GROWTH OF POPULATIONS OF THE FLOUR BEETLE, *TRIBOLIUM CONFUSUM*, DUV.

III. THE EFFECT UPON THE EARLY STAGES OF POPULATION GROWTH OF CHANGES IN THE NUTRITIVE VALUE, PALATABILITY AND DENSITY OF PACKING OF THE FLOUR MEDIUM[1]

By John Stanley[2]

Abstract

The questions of the nutritive value of the flour and its palatability have been considered mathematically, and it is shown that the number of eggs present at a time T, between the start of the culture and the time of hatching of the first egg, is decreased when the flour is less nutritious or less palatable, or less tightly packed, and *vice versa*. It is further shown that the asymptotic value ξ toward which the egg population moves in the absence of hatching, is similarly affected.

The question of the mechanism underlying these effects is discussed.

Introduction

In a recent paper, Park (1) has shown from data experimentally obtained that the use of "conditioned" flour, *i.e.*, old flour which has been contaminated with frass, etc., by being used as a medium for growing *Tribolium* beetles for prolonged periods, results in fewer eggs in comparison with the numbers in cultures made up with fresh flour. Park indicates that this result may be due to several causes, *viz.*, the accumulation of excretory and other refuse products in the flour, the depletion with age of the food content of the populated flour and the possibility of increased cannibalistic egg-eating rates due to an effect of the conditioned flour on the beetles.

It is of interest to examine these results mathematically to see the underlying mechanism which brings about these changes.

Effects Due to Changes in the Nutritive Value and Palatability of the Flour

Effect upon the Number of Eggs Existing at a Time T

In a previous paper (2) the writer has shown that the number of eggs (N_2) existing in a *Tribolium* culture at a time T, $t_0 = t_2(1) < T < t_3(1)$, *i.e.*, during the interval from the start of the culture to the instant of hatching of the first egg to hatch is,

$$T = \frac{c}{a} N_2 + \frac{(ad - bc)}{a^2} \log_e \left(\frac{aN_2 + b}{b} \right) \tag{1}$$

where

$$a = R_e N_{11} P_{11\cdot 2} A_2 W_2 r_{11\cdot 2}^2 - E_{11} P_{11\cdot 2} r_{11\cdot 2}^2 N_{11} = P_{11\cdot 2} r_{11\cdot 2}^2 N_{11} (R_e A_2 W_2 - E_{11}) .$$
$$b = R_e N_{11} P_{11\cdot 1} A_1 W_1 K_{11} r_{11\cdot 1}^2 G .$$
$$c = P_{11\cdot 2} A_2 W_2 r_{11\cdot 2}^2 .$$
$$d = P_{11\cdot 1} A_1 W_1 K_{11} r_{11\cdot 1}^2 G .$$

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Contribution from the Department of Biology, Queen's University, Kingston, Ontario, Canada.

[2] *Assistant Professor of Biology, Queen's University.*

and

R = sex ratio, *i.e.*, ratio of females to total.
ϵ = number of eggs laid per female per unit time.
N_{11} = number of adults at a time T.
N_2 = number of eggs at a time T.
$P_{11\cdot2}$ = preference of adults for eggs as a food.
$P_{11\cdot1}$ = a similar parameter for flour.
A_2 = percentage of assimilable nutrient material in eggs.
A_1 = a similar parameter for flour.
W_2 = weight of an egg.
W_1 = density of uncompressed flour.
$r_{11\cdot2}$ = radius of perception of an adult for an egg.
$r_{11\cdot1}$ = a similar parameter for flour.
G = volume of flour.
E_{11} = maintenance ration of adults, *i.e.*, the quantity of nutrient material which must be ingested per unit time by each adult to maintain the beetle's normal rate of metabolism and provide food for normal egg production.

From Equation (1) it can easily be shown that

$$\frac{\partial N_2}{\partial A_1} = \frac{\dfrac{(ad-bc)N_2}{A_1(aN_2+b)} - \dfrac{(ad-bc)}{a^2A_1}\log_e\left(\dfrac{aN_2+b}{b}\right)}{\dfrac{c}{a}+\dfrac{ad-bc}{aN_2+b}} \tag{2}$$

If, as is assumed is the case, $N_2 < -\frac{b}{a} = \xi$ where ξ is the asymptotic value towards which the egg population moves in the absence of hatching, $(ad-bc)<0$, $\log_e\left(\frac{aN_2+b}{b}\right)<0$ and $\frac{c}{a}<0$. Hence all terms on the right of Equation (2) are negative and so the right side as a whole is positive, and $\frac{\partial N_2}{\partial A_1}>0$.

From this it follows that if two cultures are started, one in old, non-nutritious flour, the other in good flour, the culture in the old flour will always contain less eggs, the assumed conditions of time and non-hatching being observed.*

Further, it is obvious that as $P_{11\cdot1}$ is always paired with A_1 in the various equations, $\frac{\partial N_2}{\partial P_{11\cdot1}}>0$, and it is thus seen that the increased number of eggs in the culture made up with good flour results partly from protection afforded the eggs by reason of the greater nutritive value of the flour, and partly from protection resulting from greater *palatability* of the flour.

In connection with the differentiation by means of which Equation (2) was obtained, an interesting point arises. Calling the right side of the equation X, for the moment one is tempted to perform the differentiation in the usual way, and write, $\frac{\partial T}{\partial A_1}=\frac{\partial X}{\partial A_1}$ where $\frac{\partial T}{\partial A_1}$ is not necessarily equal to zero, by reason of the fact that as N_2 is a function of A_1 and T is a function of N_2, then T is also a function of A_1. However, one must realize that the variable T differs from the usual variable, such as the amount of gas in a liquid-gas

**As a matter of fact, it can be shown that the assumption as to non-hatching is unimportant, but the proof from the complicated equations obtaining when $T>t_3(1)$ is uninviting.*

system, subject to changes in pressure, etc. In the case under discussion, T may be thought of in two ways, first as a true variable, namely the "time at which a certain number of eggs, N_2, is found in the population" (N_2 being an ordinary variable assuming various values), and second, as true "Time", increasing steadily, and quite independent of A_1 or any other variable in the system.

As a matter of notation, T appears in the equations as if it were thought of in the first way, but if one sets up a series of populations with various constant values of A_1 and then examines them after the same time interval has passed in each case since their initiation, then T falls into the second class, as a completely independent variable, in particular, independent of A_1 and so $\frac{\partial T}{\partial A_1} \equiv 0$.

Critical Value of A_1

From (2, Equation 27, p. 648) we have,

$$N_2' = \frac{aN_2 + b}{cN_2 + d} \quad . \tag{3}$$

Equation (3) may be written in the form

$$N_2' = \frac{\alpha + \beta A_1}{\gamma + \delta A_1} \quad . \tag{4}$$

Clearly, then, when $N_2'=0$. and the culture is at a standstill, $A_{1(0)}$ the critical value of A_1 is

$$A_{1(0)} = -\frac{\alpha}{\beta} = \frac{P_{11\cdot2} r_{11\cdot1}^2 N_{11}(E_{11} - R\epsilon A_2 W_2)}{R\epsilon N_{11} P_{11\cdot1} W_1 A_1 K_{11} r_{11\cdot1}^2 G} \quad . \tag{5}$$

Where $(E_{11}-R\epsilon A_2 W_2)$ may be written as Ξ, this being the amount of nutrient material per unit time which must be ingested to maintain metabolism and movement, etc., apart from forming and laying eggs.

From this it follows that the greater the preference for eggs, the greater the number of adults, the greater the radius of perception for eggs, and the greater the amount of energy expended in movement, the more nutritious must be the flour, if the culture is to progress*.

Further, from an examination of the denominator, it is seen that the greater the sex ratio (ratio of females to total), the more eggs laid per female per day, the greater the preference for flour, the more densely the flour is packed, the more the flour will compress as the beetles move through it, the greater the radius of perception for flour, and the *larger the culture*, the more the flour can be allowed to deteriorate without bringing the culture to a standstill.

If, of course, the food value of the flour is reduced to zero, energy slowly leaves the system through respiration and heat loss (operating through Ξ) until the beetles die, ξ being reduced to zero. This of course is analogous to the fallacy of attempting to rear chickens by feeding them on their own eggs.

**The weight and per cent of nutritive material in an egg are not included as these are probably very constant at constant temperature.*

Direct Examination of Cannibalistic Egg Eating

One may examine the problem from another viewpoint by considering cannibalistic eating directly. From (2, Equation 24, p. 648) the rate at which eggs are consumed by the N_{11} adults is

$$C'_{11.2} = \frac{E_{11} P_{11.2} r^1_{11.2} N_{11} N_2}{P_{11.2} A_2 W_2 r^1_{11.2} N_2 + P_{11.1} A_1 W_1 K_{11} r^1_{11.1} G} \tag{6}$$

from which it is at once seen that cannibalistic eating increases with a decrease in either the palatability of the flour ($P_{11.1}$) or its nutritive content (A_1). One would not expect this change to occur instantaneously when poor flour is substituted for good flour because the body of the beetle has certain reserves, and some mitigation of impending starvation can be obtained by reduction in the numbers of eggs laid (see below) and these will act as damping influences on any sudden attack on the eggs.

If A_1 be too low, E_{11} must inevitably be reduced, though it cannot fall below or even to Ξ if the culture is to continue to exist. The value of $A_{1(0)}$ will depend then to some extent on how much E_{11} is reduced through reduction in egg laying.

Inasmuch as $\epsilon A_2 W_2$ is relatively large in proportion to $A_{11} W_{11}$, it is probable that some such reduction in E_{11} occurs very soon after the beetles are placed in flour of low nutritive value, and it is suggested that this may explain the more immediate fall in egg numbers observed by Park (1) on placing beetles in conditioned flour, the later cumulative effect being due to increased cannibalistic eating in the endeavor to keep the rate of food intake above Ξ in the face of falling A_1.

Effect of a Decrease in Nutritive Value of the Flour on ξ

From (2, Equation 30, p. 649)

$$\xi = -\frac{b}{a} = \frac{R\epsilon N_{11} P_{11.1} A_1 W_1 K_{11} r^1_{11.1} G}{E_{11} P_{11.2} r^1_{11.2} N_{11} - R\epsilon N_{11} P_{11.2} A_2 W_2 r^1_{11.2}} \tag{7}$$

which may be written in the form

$$\xi = \frac{\alpha A_1 P_{11.1}}{\beta} \,. \tag{8}$$

Clearly then ξ decreases with a decrease in the palatability or nutritive value of the flour.

Changes Resulting from Denser Packing of the Flour

While it is not contended that the conditioned flour used by Park (1) is appreciably more or less densely packed than normal flour, it is of interest to examine at this point the effect of changes in the degree to which the flour is packed down, on the population.

If the flour be packed more tightly, the volume G being kept constant, it will be seen from Equation (6) that cannibalistic eating will decrease, and thus the number of eggs present at a given time will increase. Moreover, the number of eggs will increase by reason of protection from eating due to the fact that the more densely packed flour is, in effect, more nutritious per unit volume. This effect could also have been shown from Equation (1)

as $\frac{\partial N_2}{\partial W_1} > 0$. Similarly it can be shown that ξ increases with denser packing.

It might be thought that this effect would be due to the fact that as W_1 is increased, μ_{11} the speed of movement of the beetles, is decreased, and so, from (2, Equation 8, p. 644)

$$M'_{11.2} = \frac{N_{11} N_2 \mu_{11} \pi r^2_{11.2}}{G}, \tag{9}$$

the number of eggs encountered per unit time, is less, and so fewer eggs are likely to be eaten. It must be remembered, however, that for small changes in μ_{11}, $M'_{11.2}$ is independent of μ_{11}.

If large changes in μ_{11} occur, the assumption made in (2, p. 645) that "a steady stream of material passing across the field of perception gives an impression proportional to the distance traveled by the stream in unit time" is probably not justified, and $C'_{11.2}$ becomes a function of μ_{11}. Thus the factor of lessened egg-finding may be important in very tightly packed flour.

Conclusions

It is seen that the findings of Park (1) regarding the reduction in numbers of eggs in populations of *T. confusum* made up in flour of low nutritive value are confirmed by mathematical analysis.

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THE CAUSE OF BOLTING IN SWEDE TURNIPS (*BRASSICA NAPUS* VAR. *NAPOBRASSICA* (L.) PETERM.)[1]

By F. H. Peto[2]

Abstract

The relation of growth temperature to "bolting" (premature seed-stalk development) in swede turnips was investigated by growing plants in a greenhouse at high and low temperatures for different periods of time at various stages of their development. It was found that an initial growth temperature of approximately 53° F. for 30 to 50 days induced incipient bolting 71 days after seeding, as indicated by a rapid increase in internode length accompanied by a retarded root development. Flowering was inhibited to a large extent by growth temperatures above 65° F. in plants that had shown incipient bolting as a result of previous low-temperature treatments. None of the plants grown continuously at high temperatures gave any indication of bolting. It may be concluded, therefore, that high temperatures favor vegetative growth while low temperatures favor sexual reproduction.

Introduction

The swede turnip, being a biennial plant, normally develops during the first season of growth a large fleshy root with a compact cluster of leaves borne on a very short neck or stalk. If it is allowed to continue growth in the second year, it produces a tall, branched top growth bearing the inflorescence and, subsequently, seed.

It occasionally happens that some plants in a crop grown from presumably pure swede turnip seed produce inflorescences and seed the first season of growth. These plants are undesirable on account of their small root size and are commonly known as "bolters". They have, however, more than a superficial resemblance to rape and on this account the grower frequently suspects them to be such and therefore criticizes the seed merchant for selling him impure seed. These complaints were frequently referred to the Seed Branch of the Dominion Department of Agriculture and at their suggestion the problem of determining the cause of this abnormal development was undertaken in the National Research Laboratories.

The appearance of these abnormal plants or bolters in a field of swede turnips may be due to any of the following four conditions: (1) an admixture of rape or swede-like rape; (2) the presence of segregates of natural crosses between any two of swede turnips, rape and swede-like rape; (3) the presence of strains of pure swede turnips with a genetical predisposition towards bolting; (4) environmental conditions which cause a tendency to seed production rather than root production.

There are numerous publications on the cause of bolting in beets, cabbage and celery, and the majority of these indicate that low growing temperatures are primarily responsible. Only one paper was found (12) which referred to

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Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa.
[2] *Biologist, National Research Laboratories, Ottawa.*

bolting in swede turnips, and this suggested early sowing in frosty seasons as the main cause. It seemed, therefore, that a study of the effect of temperature on growth habit would provide valuable information, and since greenhouse facilities were available for a controlled experiment this phase of the investigation was undertaken.

Literature Review

Lafferty (12) ascertained the nature of certain "rogues" found among crops of swede turnips in Ireland by selecting representative specimens and inbreeding them. In addition to true yellow-fleshed swede turnips of the green- and purple-top varieties, he identified typical rape and segregates of natural hybrids between swede turnips and rape. Lafferty claimed that these rogues were either the result of an admixture of rape seed with subsequent natural crossing with swede turnips or the result of cross fertilization between adjacent fields of rape and swede turnip. He also recognized that bolters occurred in the swede turnip crop that were not due to the above causes but rather to unusual environmental conditions. He suggested that their occurrence was intimately bound up with, if not entirely due to, unduly early seeding and the incidence of late frosts which tended to check the growth of the young plants. Lafferty claimed that the above two types of bolters should be unmistakably distinguished by certain morphological characteristics. The yellow colored flesh and the buff or "Naples yellow" colored flowers of the swede should readily distinguish the environmental bolters from the natural hybrids which usually exhibit the rape characteristics of "lemon yellow" flower color and white fleshed roots.

A valuable contribution to the knowledge of color inheritance of the various organs of swede turnips was made by Davey (6, 7) and his findings in this regard should be useful in guarding against contamination of swede crops by rape or swede-like rape.

Catcheside (3) studied the chromosomal relationships in the swede and turnip groups of *Brassica*. He found that a close relationship between swedes, swede-like rapes, and bulbless bolters was indicated by their having the same chromosome number ($2n=36$), and that this close relationship was further emphasized by the conditions at meiosis. All the three groups of plants and their F_1 hybrids usually formed 18 bivalents at meiosis, showed similar degrees of secondary pairing, had approximately the same chiasma frequency at mid-diakinesis, and all formed 100% good pollen. The bulbless bolter which was studied by Catcheside was probably either a swede-like rape or a hybrid similar to those observed by Lafferty (12).

Though very little information is available on the influence of environmental factors on the incidence of bolting in swede turnips, we may form some estimate of their probable effect by a consideration of work done with other crops.

Gassner (10) studied the effect of temperature on the incidence of flowering in *Beta vulgaris* L., *Brassica oleracea* L., *Brassica Rapa* L. (fall turnip),

Brassica napus L. (winter rape), and others. Seed of these plants was sown in October and November in the greenhouse at 20° C. and grown at this high temperature until January 3. The plants were then divided into two series, one placed in the cold house at near the natural winter temperature, the other left in the warm house at about 20° F. In the middle of April all the plants were placed in the open. Only those plants which were in the cold house during the winter flowered during the following summer.

Miller (13) found that a relatively low temperature during the early stages of growth stimulated seed-stalk development in the cabbage. He tried the effect of increasing the length of day five hours by using electric light during the short days of fall and winter but found that this had no effect on seed-stalk development either in the young plants or the mature cabbage. Boswell (1) also found that the earlier cabbage was sown the greater the tendency to go to seed. In a later publication (2) he concluded that a period of exposure to low temperature was requisite for flower formation and that this low temperature was effective in promoting reproduction only after the plant had accumulated a sufficient weight of reserve foods. This accounted for the observation that seed-stalk formation was closely associated with the size of the plant at transplanting time. Heavy applications of nitrogenous fertilizer increased the size of the plant at the time of transplanting and the larger plants were observed to produce seed stalks sooner than the smaller plants. Attempts were made by Boswell to determine whether a correlation existed between the C : N ratio and the tendency of cabbages to shoot to seed. The initial experiments showed an apparent positive correlation. Subsequently an attempt was made to lower the C : N ratio by a heavy application of nitrogenous fertilizer and thereby prevent premature seed-stalk formation. The results showed that the apparent correlation between chemical constitution and the tendency to shoot to seed was a chance parallelism since, when the C : N ratio was reduced, the expected reduction in the number of seeders was not obtained.

Thompson (16, 17, 18) found that young celery plants subjected to relatively low temperature (40° to 50° F.) for two weeks or longer were likely to seed prematurely. However, growing temperatures above 70° F. prevented seeding, regardless of the previous low temperature treatment. He also found that a serious check in growth by freezing or drying tended to delay rather than hasten seed setting. Crowding of the plants had no positive effect on seed-stalk formation.

The recent findings of Chroboczek (4, 5) with reference to seed-stalk formation in beets have perhaps the closest relation to the present investigation of any of those described. His experiments also have an advantage over most of the previous work, in that he studied simultaneously the effect of temperature and light. He found that all the plants grown at 50 to 60° F. went to seed in the first year, while at the medium temperature (60 to 70° F.) only a few plants went to seed under the influence of the long day in the late spring months. At a high growing temperature all the plants developed

vegetatively. He also found that a treatment period of 30 days or longer at 40–50° F. induced flowering if the plants were subsequently grown at 60 to 70° F. under long day conditions. If, however, they were grown at temperatures of 70–80° F. subsequent to treatment, the effects of the previous cold treatment were nullified. Chroboczek also made the interesting discovery that the effect of low temperature treatment on seed development seemed to be localized in the region of the growing point. Premature seeding was induced in plants grown at relatively high temperatures by cooling only the region of the growing point to about 57° F. Another experiment by this author proved the importance of a long light duration to seed-stalk formation. When germination was commenced on January 15, seed-stalk formation had begun in one lot under continuous illumination on March 5, but under conditions of natural daylight, seed-stalk development did not commence until April 23. Munerati (14) was able to produce five generations of beets within one year by supplementing the daylight by light of high intensity during the night. Under these conditions seed stalks formed on seedlings with only two leaves. This author unfortunately fails to give any information about the other environmental factors in his unique experiment.

Garner and Allard (8, 9) studied photoperiodism in a large number of plants and came to the conclusion that a condition of balance exists between reproduction and vegetative activity. Plants were classified as long- or short-day plants according to their reaction under different lengths of day. It was found in one of their experiments (8) that a variety of soy beans required only 23 days to flower when receiving five hours of daylight while 62 days were required when the daily exposure was increased to 15 hr. Another experiment by the same authors (9) showed that reducing the growth temperature from approximately 72° to 55° F. delayed or inhibited flowering in soy beans. They also proved, for the plants they studied, that the duration of daylight rather than intensity was the important factor in exerting a controlling influence on the attainment of the reproductive stage. A reduction in intensity to one third or one fourth of normal sunlight did not modify the plant behavior to any extent. A brief reference was made by Garner and Allard to bolting in beets. They stated that the beet ordinarily is a biennial in the latitude of Washington, but that it completes its life cycle in a single season when grown in Alaska where the summer days are very long.

Very little is known of the relation of heredity to bolting in swede turnips. Differences have been observed, however, in the frequency of bolting between varieties grown under the same environmental conditions. There is an unconscious selection of non-bolters in agricultural practice, since the seed from bolters is never used. Bolters, however, have never been eliminated and the reason for this would appear to be that strains have never been selected consistently under conditions of temperature and light which have been favorable for bolting.

Thompson (18) found wide differences between varieties and strains of celery in respect to bolting, but these genetical differences were frequently

masked by the effects of environmental conditions. Van Heel (19) inbred strains of sugar beets with few and many bolters. From his results from crossing these strains he concluded that the factors for bolting were recessive. Sutton (15) crossed a green non-bolting variety of cabbage with a red bolting variety and obtained a segregation which approximated the Mendelian 9 : 3 : 3 : 1 ratio, green color and bolting being the recessive characters. Sutton, however, realized that seasonal and cultural factors were capable of influencing the frequency of bolting.

Hall (11) published the results of researches carried out at John Innes Horticultural Institution by Professor Bateson who, by inbreeding and selection of sugar beets and mangolds under conditions favorable for bolting, was successful in almost eliminating this characteristic. Hall does not believe that high resistance to bolting is either dominant or recessive, the evidence showing rather that bolting is governed by several factors. The degree of stimulus necessary to cause bolting differs with varieties and this character is inherited. The ordinary seed stock is supposed to consist of a mixed population, the members of which may respond differently to a given environment.

Methods

A greenhouse experiment was designed to test the relation of various growth temperatures to the incidence of bolting. The plants were exposed to eleven different treatments during a period of 50 days from the date of seeding. The mean temperatures in the two greenhouse rooms employed are shown in Fig. 1, the points being calculated from mean bi-hourly temperatures read from thermograph records. For the 50-day period they averaged roughly 53° and 70° F. The post-treatment temperatures of the cold and warm rooms are shown in Fig. 2. Here the temperature differential becomes progressively less, as the onset of warm spring weather made the maintenance of low temperatures impracticable. The average differentials for the treatment and post-treatment periods were thus about 17° and 7° F.

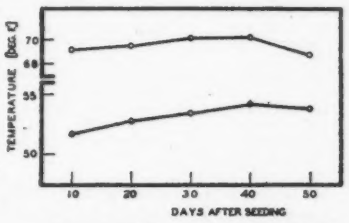


FIG. 1. *Mean temperatures in the cold and warm rooms during the treatment period.*

On the completion of the treatment period, one half of the replicates of each treatment were maintained in the warm room and the other half in the cold room. The designation of post-treatment in the cold or warm room in the diagrams, therefore, refers only to the 100-day maintenance period subsequent to the 50-day treatments, the latter being designated by the numbers 1 to 11, the key to which is given later.

Two varieties of swede turnips were used, namely, Variety A "Hall's Westbury" and Variety B "McDonald's Perfecta". Twenty replicates were placed in each treatment, making 220 plants of each variety on test. The

seeds were sown in 4-in. pots, three seeds per pot, and later thinned to single plants. At the end of the 50-day treatment period all the plants were repotted into 6-in. pots and left until the completion of the experiment. The location of the replicates on the bench was randomized to compensate for border effect and varying intensities of illumination. A different random distribution had to be calculated for each change of treatment.

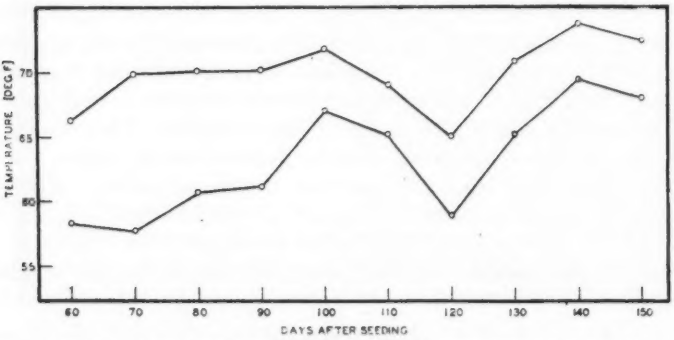


FIG. 2. *Mean temperatures in the cold and warm rooms during the post-treatment period.*

The experiment was commenced on January 21, 1934, and terminated on June 21. Normal daylight was supplemented by artificial light for eight hours from January 21 to April 5. On the latter date the artificial illumination was reduced to five hours and on May 1 it was eliminated. It was necessary to add 5 gm. of chemical fertilizer to each pot on May 21 in order to compensate to some extent for the rapid depletion of fertility due to the crowded condition of the root systems in small pots. This fertilizer consisted of superphosphate 10 parts, nitrate of soda 4 parts, muriate of potash 3 parts.

The date of emergence of the seedlings in each treatment was recorded and the stage of plant development was noted every 10 days during the treatment period by recording the number of leaves per plant. The incipient stages of bolting were indicated by a rapid increase in internode length and a slow increase in root size in certain treatments while the converse was true of the remainder of the treatments. Measurements of internode length and root diameter were taken when this differentiation was first observed. On the completion of the experiment the weights of the roots and tops as well as the internode length were recorded.

The various conditions for the 50-day treatment period are indicated in Table I.

TABLE I

Treatment no.	Description
1	High temperature for 50 days
2	Low temperature for 10 days, high for 40 days
3	Low temperature for 20 days, high for 30 days
4	Low temperature for 30 days, high for 20 days
5	Low temperature for 40 days, high for 10 days
6	Low temperature for 50 days
7*	High temperature for 10 days, low for 10 days, high for 30 days
8*	High temperature for 20 days, low for 10 days, high for 20 days
9*	High temperature for 30 days, low for 10 days, high for 10 days
10*	High temperature for 40 days, low for 10 days
11	High temperature for 50 days, with the exception of 16 hr. at 0.1° C. after 25 days' growth

**The plants in Treatments 7 to 10 were also exposed for 16 hr. at 0.1° C. on the fifth night of the low temperature treatment.*

Observations

In the low-temperature room it required an average of 15 days for Variety A and 17 days for Variety B to reach the stage where 50% of the plants had emerged. In the high-temperature room it required only 6 and 7 days respectively for Varieties A and B to reach this stage.

The growth during the 50-day treatment period was of course greater in the plants which had been grown at the high temperature for the longest period. For example, the plants in Treatment 1 had reached the 8- or 9-leaf stage, while the plants in Treatment 6 had only reached the 6-leaf stage at the end of the treatment period. An exposure of 10 days at the lower temperature had no noticeable effect on plant growth regardless of the stage of growth at which this exposure was made (Treatments 2, 7, 8, 9 and 10). The plants in Treatments 4, 5 and 6 which were placed in the cold room for 30, 40 and 50 days respectively were appreciably smaller than the remainder at the end of the treatment period.

The first evidence of any tendency to bolting was observed 21 days after the completion of the treatment period. The plants of the four most prolonged cold treatments (20, 30, 40 and 50 days) were noticeably different from the remainder in that the internodes had begun to lengthen and there was little or no increase in root diameter. In comparison, the plants grown for longer periods at the higher temperature had commenced to develop normal roots and there was no pronounced elongation of the internodes. At this time measurements of the root diameter of all the plants were taken at the point of greatest enlargement above the surface of the soil. Measurements of the total internode length were also made. It was considered that the internode length divided by the root diameter would give a quotient which would be indicative of the bolting tendencies of the plants so measured. The main advantage of this quotient is that it presents a picture of the relative development of root bulb and stalk regardless of the size of the plant

which, of course, is also influenced by the treatment. The significance of the treatment differences so obtained was tested by the calculation of the standard error.

TABLE II

MEAN OF TOTAL INTERNODE LENGTH DIVIDED BY ROOT DIAMETER
(71 days after seeding)

Treatments	Variety A, post-treatment in warm room		Variety B, post-treatment in warm room		Variety A, post-treatment in cold room		Variety B, post-treatment in cold room	
	Mean	S.E. of M.	Mean	S.E. of M.	Mean	S.E. of M.	Mean	S.E. of M.
1	1.52	± 0.15	2.08	± 0.43	1.45	± 0.18	1.50	± 0.21
2	1.82	± 0.23	1.94	± 0.25	1.50	± 0.16	1.42	± 0.09
3	2.40*	± 0.33	3.15	± 0.83	2.76*	± 0.48	2.12*	± 0.15
4	4.46*	± 1.00	4.20*	± 0.66	3.04*	± 0.52	2.98*	± 0.36
5	4.30*	± 0.53	4.88*	± 0.85	3.21*	± 0.42	3.15*	± 0.41
6	4.70*	± 0.80	5.62*	± 1.13	3.70*	± 0.46	3.10*	± 0.36
7	2.06*	± 0.20	1.68	± 0.13	1.62	± 0.29	1.66	± 0.19
8	1.94	± 0.30	1.64	± 0.15	1.95	± 0.49	1.33	± 0.18
9	1.77	± 0.14	1.46	± 0.13	1.28	± 0.18	1.45	± 0.30
10	1.50	± 0.18	1.88	± 0.35	1.80	± 0.15	1.23	± 0.12
11	1.87	± 0.19	1.31	± 0.13	1.29	± 0.16	1.16	± 0.10

**Mean of treatment significantly higher than that of Treatment 1.*

According to Table II the quotients calculated for Treatments 3, 4, 5 and 6, which included an initial period of 20, 30, 40 and 50 days respectively in the cold room, were all higher than those for Treatment 1, significantly so with the one exception of Treatment 3, Variety B, post-treatment in warm room.

The only other quotient significantly higher than the corresponding value in Treatment 1 is that for Treatment 7, Variety A, post-treatment in warm room. This is the only instance where 10-day low-temperature treatment induced any tendency to bolt and it will be noticed from later records that this difference was subsequently eliminated.

The high ratio of internode length to root diameter is considered to be definite evidence of incipient bolting. The pictures in Plate I were taken two weeks after the observations were made and they show the differences in type of development between typical plants of the 11 treatments. The lack of root development and the long internodes can be seen in the plants of Treatments 4, 5 and 6.

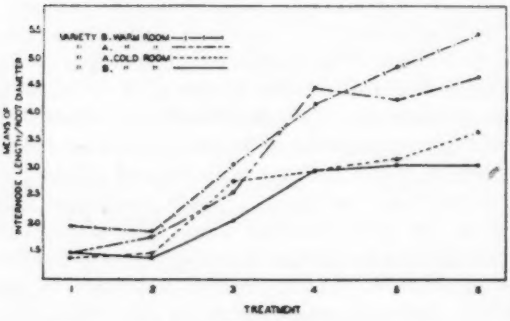
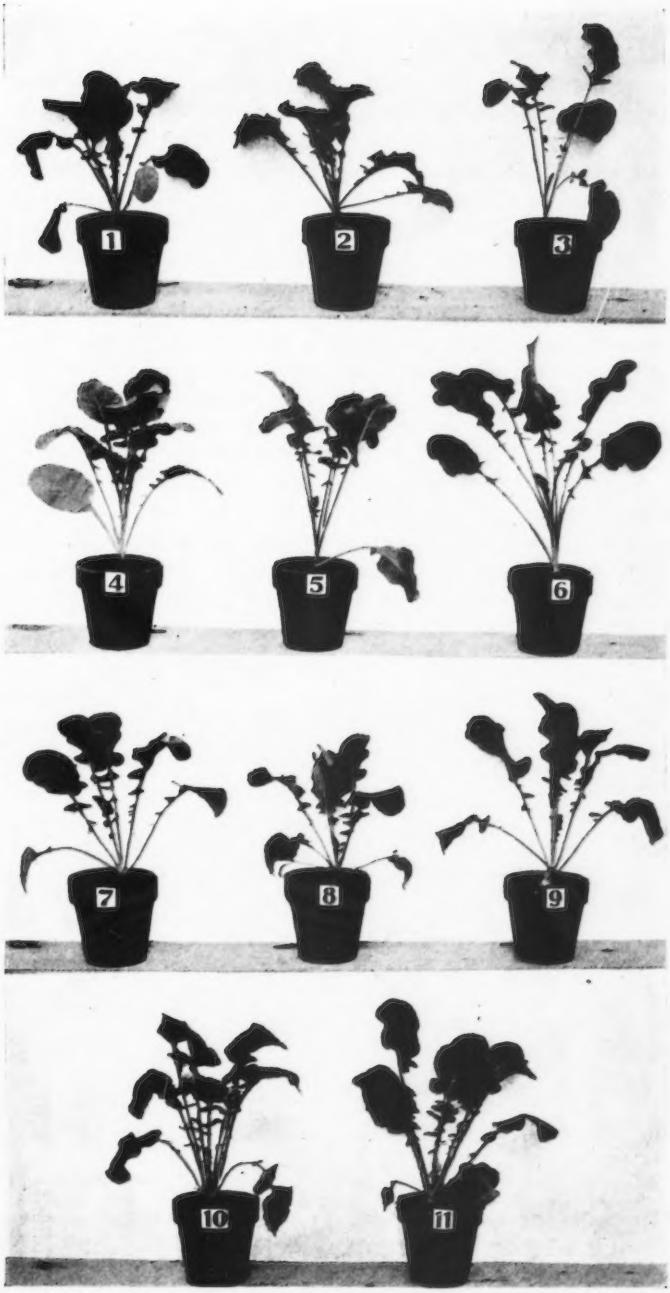


FIG. 3.



Variety B. *Representative plants of treatments 1 to 11, 86 days after seeding.*

Fig. 3 illustrates the differences brought out by statistical analysis of Treatments 1 to 6. The fact that a tendency to bolt can be detected thus early in development does not necessarily indicate that these plants will bolt regardless of subsequent environmental conditions. A high maintenance temperature may check seed-stalk development and initiate storage-root development. Some evidence of this was observed at a later stage of development when plants which had shown a very marked elongation of internode length during earlier development at lower temperatures, began to show a decreased internode length between freshly formed leaves, and additional root development, after being exposed to higher temperatures.

Inflorescences appeared on three plants of Treatment 6, Variety A, and two plants of Treatment 6, Variety B, between 99 and 135 days after seeding, where the plants were subjected to the post-treatment in the cold room. Under conditions where a higher maintenance temperature was employed after treatment only one plant came into flower. This was found in Treatment 6, Variety A, 130 days after seeding. This difference indicates the sensitivity of these plants to comparatively small temperature differences, as there was an average temperature difference of only 5° F. between the low- and high-temperature rooms during the flowering period.

The experiment was terminated on June 21, since it was apparent that no more plants would flower under the high temperatures which existed in both rooms at this time. The effect of the treatments on the plant type nevertheless could be clearly seen at this time as illustrated by the contrast between the plants in B and D, Fig. 4. The plant C has not produced an

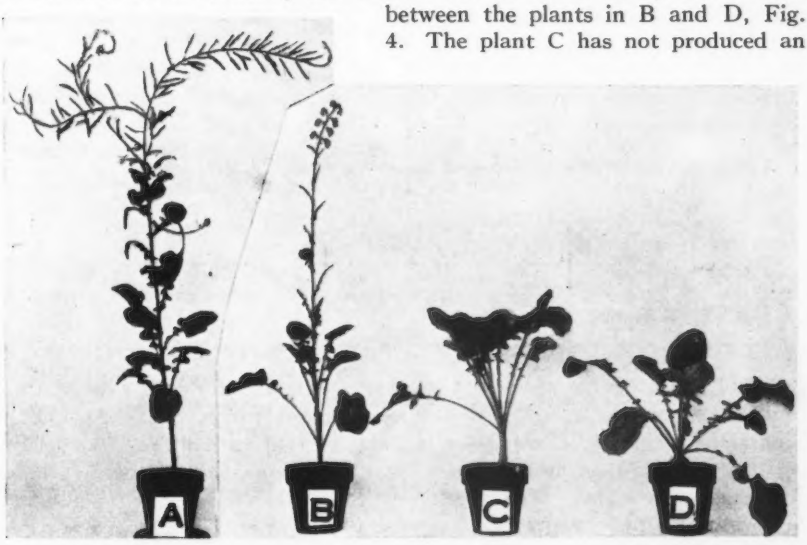


FIG. 4. *A. Variety* B. *150 days after seeding. Treatment 6 followed by growth in cold room. B. Same plant, 113 days after seeding. C. Variety* B, *113 days after seeding; Treatment 6 followed by growth in warm room. D. Variety* B, *113 days after seeding; Treatment 11, followed by growth in warm room.*

inflorescence but it can be definitely classified as a bolting type. Observations were taken at the completion of the experiment on root and top weight, internode length, and the ratio between root weight and internode length.

TABLE III

FINAL MEAN ROOT WEIGHT

(5 months after seeding)

Treatment	Variety A, post-treatment in cold room, gm.	Variety B, post-treatment in cold room, gm.	Variety A, post-treatment in warm room, gm.	Variety B, post-treatment in warm room, gm.
1	75.2	94.2	61.5	62.3
2	71.4	68.9	52.8	84.9
3	60.4	63.1*	47.0	76.0
4	40.9*	78.3	34.4*	44.0
5	25.9*	40.2*	22.0*	37.2
6	34.5*	53.9*	36.4*	42.2
7	61.0	92.3	59.4	84.4
8	61.0	83.1	56.9	81.4
9	61.3	96.0	48.2	79.6
10	113.6	130.2	74.5	117.5
11	68.2	78.7	65.1	102.9
S.E. of difference between two treatment means	±10.5	±14.1	±9.8	±13.8

**Mean root weight significantly lower than that of Treatment 1.*

TABLE IV

RESULTS OF ANALYSIS OF VARIANCE OF ROOT WEIGHT

Variety	Post-treatment room	Variance between treatments	Variance within treatments	S.E. of one treatment mean, gm.	S.E. of difference between two treatment means, gm.
A	Cold	5,189.23	547.11	7.4	10.5
B	Cold	5,817.12	989.83	10.0	14.1
A	Warm	2,322.80	480.96	6.9	9.8
B	Warm	6 446.72	951.66	9.8	13.8

Table III gives the mean root weights of 10 plants for each variety, treatment and post-treatment. The results of an analysis of variance of the 220 individual root weights are shown in Table IV. In spite of the high variation within the treatments it is apparent that the variance between the treatments is significantly higher. The calculation of the standard error of the difference between two treatment means permitted an examination of the mean root weights to determine which of the temperature treatments had significantly depressed or increased the root yield over Treatment 1 (the plants of which were kept in the high-temperature room at a mean temperature of approxi-

mately 70° F. throughout the treatment period). The asterisks in Table III indicate the mean root weights that were significantly lower than that of Treatment 1. For Variety B, post-treatment in warm room, none of the root yields were significantly lower than in Treatment 1, the statistical comparison being influenced by one abnormally small root in Treatment 1. However, the mean of Treatments 4, 5 and 6 is significantly lower than the mean of Treatments 1 and 2, the values being: mean of Treatments 4, 5 and 6=41.1 gm.; mean of Treatments 1 and 2=73.6 gm.; standard error of the mean difference between two and three treatments =8.49 gm. The data in Table III, as well as Figs. 5 and 6, indicate that an initial mean growth temperature of approximately 53° F. for 30, 40 and 50 days (Treatments 4, 5 and 6) has a depressing effect on yield. The top weights, on the other hand, were maintained or even increased under these conditions.

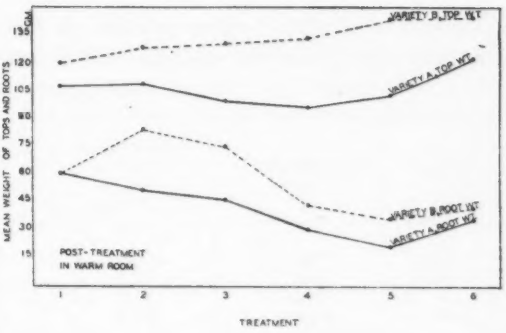


FIG. 5.

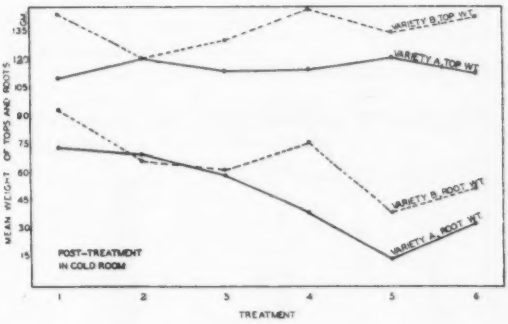


FIG. 6.

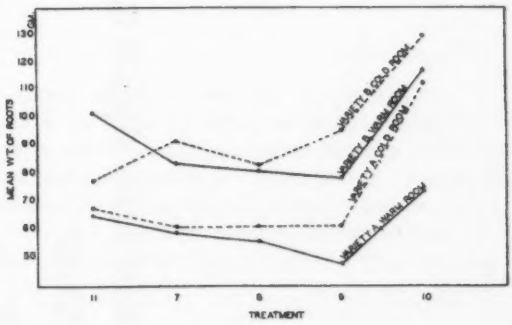


FIG. 7.

In Treatments 7, 8, 9 and 10 the plants were given 10 days in the cold room when 10, 20, 30 and 40 days old respectively. The root weights are shown in Fig. 7 and indicate that a low-temperature treatment after 40 days in the warm room (Treatment 10) stimulates root development, while the same treatment at earlier stages has no significant effect. When

Treatments 7, 8 and 9 were grouped, it was found that the mean of Treatment 10 was significantly higher in all cases as shown in Table V.

TABLE V

Variety	Post-treatment room	Mean root weight of Treatments 7, 8 and 9, gm.	Mean root weight of Treatment 10, gm.	S.E. of difference between mean of one and three treatments, gm.
A	Cold	61.1	113.6	± 8.1
B	Cold	90.3	130.2	±11.0
A	Warm	54.8	74.5	± 7.6
B	Warm	81.8	117.5	±10.7

TABLE VI

FINAL MEAN INTERNODE LENGTH

(5 months after seeding)

Treatments	Variety A, post-treatment in cold room, cm.	Variety B, post-treatment in cold room, cm.	Variety A, post-treatment in warm room, cm.	Variety B, post-treatment in warm room, cm.
1	4.1	5.0	4.9	6.7
2	5.5	4.6	5.5	4.8
3	7.0	5.8	6.1	7.6
4	11.6	9.3	9.0	6.8
5	10.9	9.7	9.8	11.2
6	39.3	35.8	15.4	21.7
7	6.1	8.1	7.3	6.0
8	7.8	6.8	6.9	3.5
9	5.5	7.2	6.9	3.5
10	8.0	5.4	5.4	5.6
11	5.6	4.2	7.0	3.5

It is of interest to note the internode length of the plants in the various treatments as shown in Table VI and Figs. 8 and 9. There appears to be a gradual increase in internode length for the most prolonged low-temperature treatments. In Treatment 6, the plants maintained in the cold room following the treatment period showed a marked increase in the mean length, owing to the number which actually flowered.

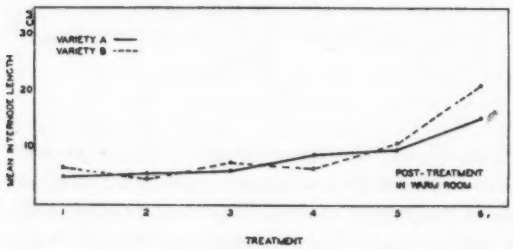


FIG. 8.

It was considered that the degree of differentiation towards bolting could be conveniently expressed by dividing the internode length by the root weight. This quotient was particularly useful since it corrected to some extent for differences in plant size

between treatments. A normal turnip or non-bolter would have a heavy storage root and would have the leaf nodes lying close together, whereas a typical bolter would lack the heavy storage root and would have a tall stalk with the nodes widely spaced. From the data shown in Table VII and also from Figs. 10, 11, 12 and 13 it can be seen that the quotients increase regularly from Treatments 1 to 6 (with two exceptions seen in Figs. 12 and 13). The tendency to bolt is thus correlated with the length of exposure to initial growing temperatures of 50° to 55° F. and increases rapidly when this exposure exceeds 30 days, as is shown by the sharp upward movement of the curve from Treatments 4 to 6.

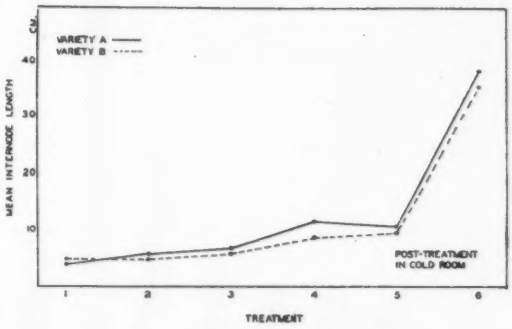


FIG. 9.

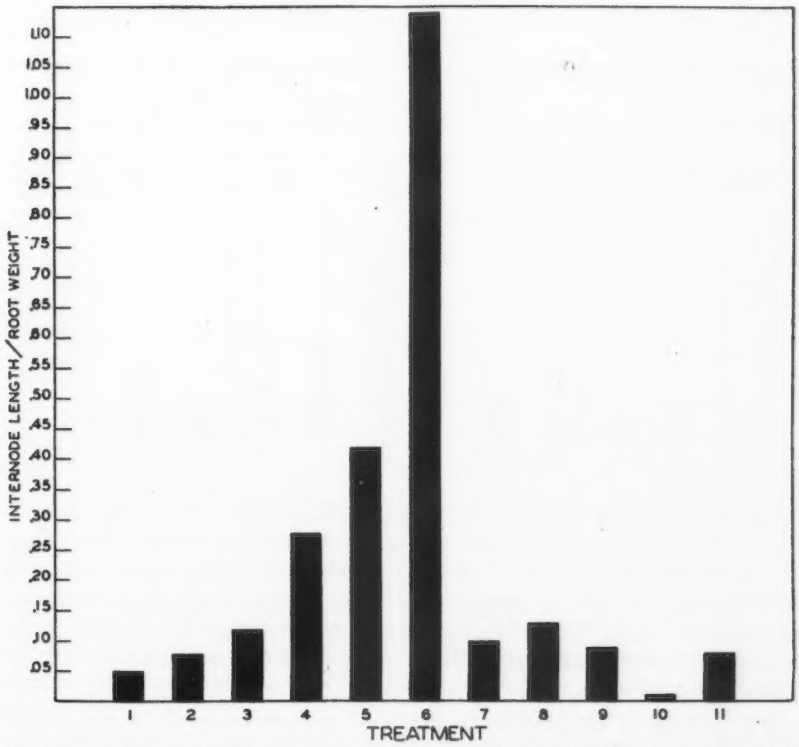


FIG. 10. *Variety* A, *post-treatment in cold room, mean internode length divided by mean root weight.*

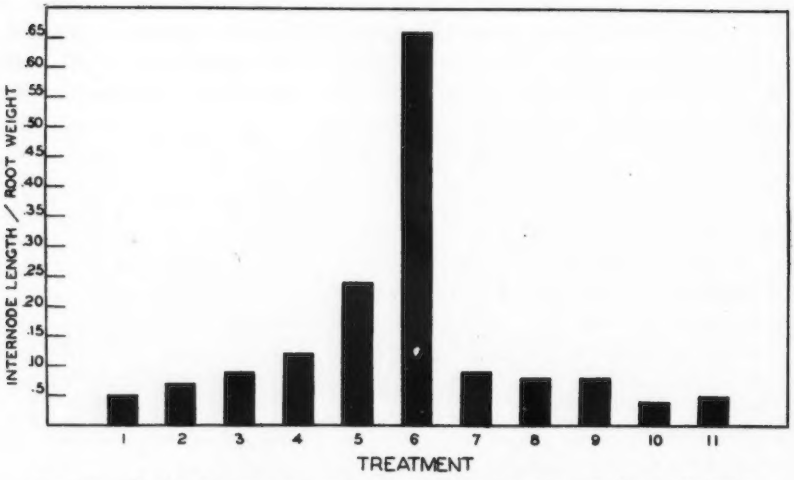


FIG. 11. *Variety* B, *post-treatment in cold room, mean internode length divided by mean root weight.*

TABLE VII

QUOTIENT OBTAINED BY DIVIDING FINAL MEAN INTERNODE LENGTH BY MEAN ROOT WEIGHT

(5 months after seeding)

Treatment	Variety A, post-treatment in cold room	Variety B, post-treatment in cold room	Variety A, post-treatment in warm room	Variety B, post-treatment in warm room
1	0.06	0.05	0.08	0.11
2	0.08	0.07	0.10	0.06
3	0.12	0.09	0.13	0.10
4	0.28	0.12	0.26	0.16
5	0.42	0.24	0.44	0.30
6	1.14	0.66	0.42	0.51
7	0.10	0.09	0.12	0.07
8	0.13	0.08	0.12	0.04
9	0.09	0.08	0.14	0.04
10	0.01	0.04	0.07	0.05
11	0.08	0.05	0.11	0.03

The post-treatment in the cold room produced more actual seeders than the post-treatment in the warm room and from the data illustrated in Figs. 10, 11, 12 and 13 it is seen that the general tendency to bolt is also more marked under the former conditions. Owing to outside weather conditions, the temperature in the "cold" room actually averaged 65.7° F. during the last 50 days of the experiment, and it may be that a lower post-treatment temperature would have resulted in a much higher percentage of true seeders.

It is interesting to note the character of the low-temperature treatments which failed to exhibit any pronounced evidence of bolting on the completion

of the experiment, *viz.*, (a) an initial cold treatment of 10 or 20 days, (b) a 10-day cold treatment after 10, 20, 30 or 40 days' growth which included 16 hr. at 0.1° C., (c) a cold treatment of 16 hr. at 0.1° C. after 25 days' growth.

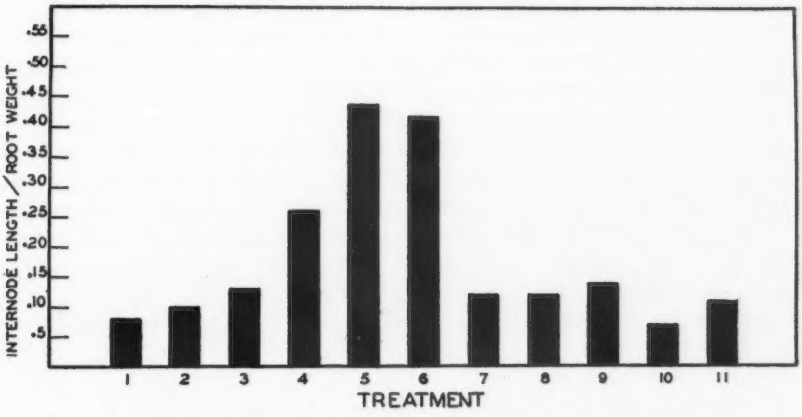


FIG. 12. *Variety* A, *post-treatment in warm room, mean internode length divided by mean root weight.*

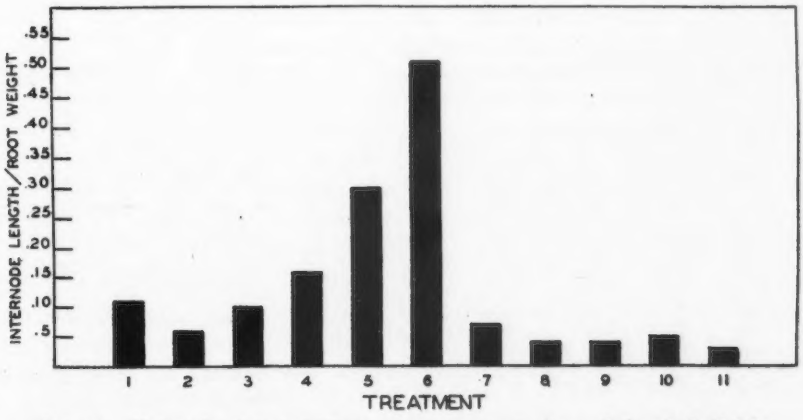


FIG. 13. *Variety* B, *post-treatment in warm room, mean internode length divided by mean root weight.*

Discussion

The question which suggests itself to the grower is whether growth temperatures which caused bolting in the greenhouse would have a similar effect under field conditions. That is difficult to answer as there are certain obvious differences in the two environments. In the first place, there is probably a higher light intensity in the field which might favor bolting. Whether intensity of light is important or not, it is almost certain that duration is, and this is

continuously changing throughout the season. Second, there are differences in the diurnal temperature variations between the two environments, on which there is no evidence. Differences in moisture and nutrients also may have a minor effect.

Considering temperature only, there is some reason for believing that with varieties with the same genetic susceptibility to bolting as the ones used in this experiment, bolting would occasionally be encountered under field conditions. A rough indication of this is afforded by a consideration of Table VIII which gives the monthly means of bi-hourly readings for May, June, July and August for the years 1918 to 1927 in Montreal. The occurrence of unusually low growing temperatures particularly in May and June may account for the occurrence of bolters in some years. For example, the mean temperature for these months at Montreal was 6.4° F. lower in 1926 than in 1921. A comparison is made of the outside temperatures at Montreal with the cold-room temperatures used in this experiment. The mean temperature during the initial 30-day period in the cold room was approximately 53° F., while the 10-year mean temperature at Montreal for May is 54.9° F. For the succeeding 90 days the mean cold-room temperature was approximately 60° F. and the mean temperature at Montreal was 63.3° F. Since the swede turnip plants grown in the cold room (Treatment 6) all showed a definite tendency to bolt, it might be concluded that the average growth temperature at Montreal would be favorable to bolting if swede turnips were sown on May 1. The usual practice, however, is to sow swede turnips about May 15 and the delay might be sufficient to inhibit bolting in the varieties in question. There would be a difference of 5.5° F. in the mean growth temperature for a three-month period, for seedings of May 1 and June 1 in Montreal. This indicates the possible relation between date of sowing and incidence of bolting.

TABLE VIII

MONTHLY MEANS OF BI-HOURLY TEMPERATURE READINGS RECORDED AT MONTREAL, QUEBEC, ° F.

Year	May	June	July	Aug.	Mean
1918	59.3	61.2	70.0	67.0	64.37
1919	55.5	68.7	70.0	65.4	64.90
1920	57.0	65.5	67.2	70.1	64.95
1921	59.6	66.6	75.4	67.1	67.17
1922	59.0	64.5	69.2	66.2	64.72
1923	53.2	65.0	66.7	64.9	62.45
1924	50.0	64.0	68.6	66.5	62.27
1925	51.0	65.6	66.6	68.8	63.00
1926	52.7	60.7	68.3	66.5	62.05
1927	52.5	61.4	68.3	64.1	61.57
Mean	54.9	64.3	69.0	66.6	

In considering the causes of bolting in turnips, the possibility should not be overlooked that these so-called bolters are in some cases actually rape, swede-like rape, or natural hybrids between either of these and swede turnips. The majority of these plants should be fairly easily distinguished by certain morphological characters not typical of the swede turnip, particularly flesh and flower color as emphasized by Lafferty (12). However, certain segregates of these natural hybrids may be hard to distinguish and a progeny test then

becomes the only positive means of identification. If the so-called bolters cannot be accounted for in this manner and appear to have all the swede turnip characters then one must seek the cause in heredity or environment.

Strains of swede turnips which are particularly susceptible to bolting undoubtedly exist, and a proportion of plants may exhibit this habit even under average growth conditions in the field. If the growing temperatures were unusually low owing either to a cold season or very early seeding, a high percentage of bolters would be expected. On the other hand it is quite probable that most of our turnip varieties have a low susceptibility to bolting and would only show an appreciable number of bolters under conditions particularly favorable to such behavior. These predictions are based on the present greenhouse experiments, the previous observations by Gassner (10) and Lafferty (12), and the experiments on closely related root crops.

Additional greenhouse and field experiments are required to clear up the numerous uncertainties in regard to the causes of bolting, but it appears to be worth while to consider the possible solution of the practical problems from the evidence at hand. The breeder's problem is to select turnips which will have a low susceptibility to bolting. This means selection of biotypes with genetical factors favoring the biennial rather than the annual habit. The chances of accomplishing this would be considerably enhanced by subjecting the breeding material at some stage to environmental factors favoring bolting. The same method should be used to eliminate bolters in recognized varieties. Additional morphological studies of off-type plants and bolters should be made to supply inspectors and growers with information which might enable them to distinguish accurately between off-types that are the result of admixture or hybridization with rape or swede-like rape, and those which are the result of true bolting in pure swede turnips. When an accurate picture of the relative importance of the various environmental factors is available, the grower may be able to make certain adjustments in his cultural practice to reduce the losses from bolting. The most promising solution, however, appears to be in breeding swede turnips which are highly resistant to bolting.

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POLARIMETRIC ESTIMATION OF STARCH[1]

By Clarence Yardley Hopkins[2]

Abstract

For the estimation of starch in wheat and certain wheat products, an adaptation of the polarimetric method of Mannich and Lenz has been developed. It gives satisfactory results with wheat, wheat flour and wheat starch and with mixtures of known composition. Proof is given that the method is accurate in the presence of proteins, sugars and hemicelluloses. It avoids the errors inherent in hydrolytic methods and is believed to be more convenient than Rask's procedure.

Introduction

The exact determination of starch in cereal products is a matter of some difficulty and the methods in common use are not entirely satisfactory with respect to accuracy and ease of manipulation.

The methods of estimating starch may be grouped into four classes: (a) acid hydrolysis, followed by estimation of glucose, (b) enzymic hydrolysis (diastase), followed by estimation of glucose, (c) isolation and weighing of starch, (d) dispersion of starch and estimation by polarimeter.

Acid hydrolysis of cereal products has been shown by Fraps (8) and others to give high results for starch, owing to the production of reducing sugars from pentosans and hemicelluloses. The apparent starch content may be in error by as much as 5% on this account.

Enzymic hydrolysis by diastase is slow and requires correction for a high blank, owing to the presence of sugars in the malt extract used. Herd and Kent-Jones (11) and Rask (17) obtained low results on pure wheat starch by the method of enzymic hydrolysis (diastase). If taka-diastase is employed the blank correction is reduced but the disadvantage of a long digestion period is still present. Furthermore, it has been shown by Herd and Kent-Jones (11) and by Denny (4) that diastatic enzymes will produce reducing sugars from substances other than starch.

The gravimetric methods (c) include those of von Fellenberg (7), Baumert (1), and Rask (17), the last having received the most attention. In Rask's method the starch is dispersed in dilute hydrochloric acid, filtered, precipitated and weighed directly. The results appear to be closer to the true starch content than those of methods (a) or (b). The procedure has been studied and revised by the American Association of Agricultural Chemists (12, 13, 16, 18) but the revised procedure is not entirely satisfactory. It has been adversely criticized by Hall (9) and by Denny (5). A combination of the Rask or von Fellenberg method with estimation by taka-diastase is recommended by Denny (4, 5).

The direct polarimetric estimation of starch was studied by Ewers (6) and by Lintner and Belschner (2). These investigators dispersed the starch in

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Contribution from the National Research Laboratories, Ottawa, Canada.
[2] *Chemist, National Research Laboratories, Ottawa.*

dilute hydrochloric acid and used various procedures to obtain a clear solution for polarization. As shown by Hals and Heggenhaugen (10) the acid dissolves certain optically active substances other than starch, which makes the results inaccurate. Furthermore, there is appreciable hydrolysis of starch by the hydrochloric acid during the period of analysis (15).

A new polarimetric method, introduced by Mannich and Lenz (15) in 1920, has received less attention than it deserves. The procedure consists in dispersing the starch in a fairly concentrated calcium chloride solution, filtering and polarizing. The authors give only a few of their analytical results and report an agreement between duplicates within about 10 parts per 1000. The present work was designed to test this method and to determine whether it is applicable to wheat and wheat products.

Mannich and Lenz' Method

The finely ground sample is weighed into a casserole, rubbed up with 10 cc. of water and treated with 60 cc. of calcium chloride solution (two parts crystalline calcium chloride to one part water) and 1 cc. of 0.8% acetic acid. The mixture is boiled gently for 15 min., cooled, filtered, made up to 100 cc. and polarized. In order to correct for the rotation due to soluble proteins and sugars, a separate sample is covered with 100 cc. of calcium chloride solution and stirred at intervals for 30 min. at room temperature. The solution is filtered and polarized. The algebraic difference of the rotations of the two samples gives the rotation due to starch alone. Alternatively, the protein may be removed from the original sample by precipitation with zinc chloride prior to the filtration.

Examination of the Mannich and Lenz Method

The first part of the procedure was found to be very satisfactory and to give concordant results. In attempting to carry out the second part (digestion at room temperature) it was found that some starch was dissolved. Even at 10° C. the filtrate in many cases gave a positive test for starch. Furthermore, it was found that wheat gluten and sucrose do not give the same rotation when dissolved in hot calcium chloride solution that they give when dissolved in cold calcium chloride solution.

Removal of the protein by zinc chloride was also unsatisfactory. The filtrate was cloudy, and there was some evidence of progressive hydrolysis of the starch.

Separation of the starch from protein was attempted by precipitating the starch from the filtrate obtained in the first part of Mannich and Lenz' procedure. Both iodine and alcohol were tried as precipitants and the recovered starch was redissolved in aqueous calcium chloride for polarization. These steps lengthened the procedure considerably, but the most serious difficulty was that precipitation seemed to be incomplete and results were low. The filtrate from the starch-iodide precipitate, although it gave no color with iodine, had a strong positive rotation which could scarcely have been due to anything but starch. One experiment indicated that this unprecipitated

starch amounted to 9% of the starch in the sample. This result is in agreement with the belief that a part of the starch grain does not give a color with iodine (14).

Revision of the Mannich and Lenz Method

It was found that the soluble proteins and sugars are best removed at the beginning by repeated washing with 65% (by weight) alcohol. After this preliminary washing the sample is boiled with calcium chloride solution, using double the amount of acetic acid recommended by Mannich and Lenz. Minor changes have been made in the remainder of the procedure.

The material removed from wheat and wheat flour by the alcohol wash has a negative rotation and in most cases would reduce the apparent starch content by 1 to 1.5% if left in the sample.

The completeness of removal of interfering proteins and of sucrose by this method was proved by the satisfactory analysis of prepared mixtures of wheat gluten and wheat starch and of sucrose and wheat starch. Sucrose has been shown to be the chief sugar present in flour (3).

Analysis of a prepared mixture of starch-free wheat straw and wheat starch showed that the hemicelluloses do not interfere.

The revised method gave excellent checks in duplicate determinations and is fairly rapid, a pair of analyses requiring about 3½ hr. Confirmation of Mannich and Lenz' conclusions was obtained with respect to the following: complete dispersion of starch by boiling for 15 min., absence of hydrolysis of starch during the analysis, and negligible change in specific rotation with temperature changes between 20° and 30° C.

The specific rotation of wheat starch in the solution used is taken as $[\alpha]_D = 200.0$, in accordance with the recommendation of Mannich and Lenz.

It is planned to continue the present work, which deals with wheat starch, to include other cereal starches and potato starch.

The Revised Method

The sample is finely ground (minus 100 mesh if possible) and 2.0 to 2.5 gm. is weighed into a 50 cc. round-bottomed centrifuge tube with lip. It is washed by adding 10 cc. of aqueous alcohol (d_{20}, 0.88) and stirring thoroughly with a glass rod. The rod is placed carefully aside while the mixture is centrifuged and the liquid poured off. The washing is repeated until 60 cc. of wash liquid has been used, stirring each time with the same rod.

The residue is stirred with 10 cc. of water and poured into a 125 cc. Erlenmeyer flask. The transference is completed by washing out with a total of 60 cc. of calcium chloride solution (see below) containing 2 cc. of 0.8% acetic acid. The rod is also transferred to the flask and the mixture is brought to boiling quickly over wire gauze, with frequent stirring. It is boiled briskly

for 15 to 17 min. while precautions are taken to prevent burning and foaming. Particles on the sides of the flask are rubbed down with the rod from time to time.

The solution is cooled quickly in running water and poured into a 100 cc. volumetric flask, rinsing thoroughly with calcium chloride solution (see below) from a wash-bottle with a medium jet. In making up to the mark, it is permissible to destroy the froth by adding one drop of alcohol.

After thoroughly mixing, about 10 cc. is poured on to a fluted filter (Whatman No. 42 or 44), wetting the paper completely. This is allowed to run dry and the filtrate is discarded. Filtration is resumed using a dry receiver, and 40 to 50 cc. is collected.

The liquid is polarized in a 10 cm. tube, taking two sets of 10 readings each. The averages of the two sets should agree within 0.006°.

The starch content is calculated as follows:

$$\text{Starch, } \% = \frac{100 \times A \times 100}{1 \times 200 \times S} = \frac{50 \times A}{S},$$

where A is the observed rotation and S the weight of the sample.

The calcium chloride solution is prepared by dissolving two parts of crystalline calcium chloride ($CaCl_2 \cdot 6H_2O$) in one part of water and adjusting to a density of 1.30 at 20° C. This solution contains about 33% of calcium chloride. It is made faintly pink to phenolphthalein by adding $N/10$ sodium hydroxide, and is then ready for use. Anhydrous calcium chloride may be employed but is usually alkaline and requires the addition of acid to bring it to the correct pH.

Experimental

Wheat Starch

The method was applied to a commercial sample of wheat starch (General Chemical Co.) and the non-starch constituents were determined at the same time.

TABLE I

COMPLETE ANALYSIS OF WHEAT STARCH

Non-starch						Starch, %	Total
Moisture, %	Sugars, %	Protein, %	Ash, %	Fibre, %	Fat, %		
10.97, 11.02 Av. 11.00	0.35	0.31	0.21	0.12	0.03	87.80, 87.85 Av. 87.83	99.85

A blank experiment showed that there was no detectable amount of starch in the reagents or the filter paper.

Wheat Flour and Whole Wheat

Wheat flour requires no further grinding. The wheat kernels were ground in a Wiley mill, with special precautions to avoid loss. Care was taken to mix the ground material thoroughly before taking a sample for analysis.

The results of the analyses, along with those for wheat starch, are given in Table II.

TABLE II

STARCH CONTENT OF WHEAT, FLOUR AND STARCH

(13.5% moisture basis)

Material	Description	Starch, %	Mean, %	Difference between duplicates, %
Wheat, hard spring	Marquis, No. 1 Northern	51.75, 52.00	51.88	0.25
Wheat, soft winter	Dawson's Golden Chaff	59.08, 59.02	59.05	0.06
Flour, hard wheat	Marquis	68.01, 68.10	68.06	0.09
Flour, soft wheat	Ben Hur	71.20, 70.97	71.09	0.23
Starch, wheat	Commercial, No. 1	85.33, 85.38	85.36	0.05
	No. 2	85.61, 85.67	85.64	0.06

Analysis of Prepared Mixtures

Mixtures of wheat starch with starch-free substances were prepared and analyzed for starch. The true content of starch in the sample of wheat starch was taken as 87.98%, which figure was obtained by deducting from 100 the sum of the impurities listed in Table I. The starch content of the mixtures is calculated from this figure.

The results, which are given in Table III, show that the method is reliable in the presence of substances which might be expected to interfere.

TABLE III

ANALYSIS OF PREPARED MIXTURES

Material	Starch, %		Error, %
	Calcd.	Found	
Wheat starch	87.98	87.83	0.15
Starch plus wheat gluten	75.82	76.02	.20
Starch plus wheat gluten	79.59	79.73	.14
Starch plus sucrose	79.50	79.39	.11
Starch plus wheat straw	79.73	79.86	.13

The gluten was prepared by washing out a sample of hard wheat flour until the washings no longer contained starch. The wheat straw, which appeared to contain originally about 0.5% starch, was de-starched before use by treatment with malt extract.

Comparison with Rask Method

A comparison of the results obtained by the polarimetric method and the Rask method was made possible through the kindness of V. E. Munsey, who supplied specimens which had been analyzed for starch by the latter procedure.

The comparative figures are shown in Table IV.

TABLE IV

COMPARISON OF POLARIMETRIC AND RASK METHODS

Material	Moisture, %	Starch, % (as received)		Difference, %
		Polarimetric	Rask	
Whole wheat flour	13.36	(56.76, 56.65) 56.71	58.28	1.57
White flour	13.70	(67.69, 67.63) 67.66	68.28	0.62

Comparison with Mannich and Lenz' Method

It was found that omission of the preliminary alcohol wash gave results as much as 1 to 2% too low, owing to the effect of soluble laevorotatory protein.

TABLE V

EFFECT OF SOLUBLE PROTEIN ON RESULT OF STARCH ANALYSIS

(13.5% moisture basis)

Material	Starch, % (mean)		Difference, %
	Without alcohol wash	With alcohol wash (see Table II)	
Wheat, hard spring	(50.41, 50.53) 50.47	51.88	1.41
Wheat, soft winter	(58.87, 58.80) 58.84	59.05	0.21
Flour, hard wheat	(66.30, 66.37) 66.34	68.06	1.72
Flour, soft wheat	(69.68, 69.70) 69.69	71.09	1.40
Starch, wheat No. 1	(85.51, 85.35) 85.40	85.36	0.04

As would be expected, the results on pure wheat starch are the same by the two methods.

Separate samples of these materials were treated with cold calcium chloride solution according to the method of Mannich and Lenz. The results were unsatisfactory owing to the fact that some of the starch was dissolved. Numerous tests were made to find conditions under which starch would not dissolve, but without success. It was found that a temperature of 20° C. or higher is quite unsuitable. Some of the results are given in Table VI.

TABLE VI

RESULT OF TREATING WHEAT AND FLOUR WITH COLD CALCIUM CHLORIDE SOLUTION

Material	Conditions	Iodine test	Rotation
Wheat	30 min. 10° C.	+	−
Wheat	30 min. 20° C.	+	+ (0.012°)
Wheat	30 min. 26° C.	+	+ (0.742°)
Flour	30 min. 10° C.	Trace	−
Flour	30 min. 10° C.	Trace	−
Wheat	After alcohol wash; 30 min. 10° C.	+	+

In further experiments it was found that changing the pH of the calcium chloride solution made a difference in the amount of starch dissolved. In one or two tests the final solution was free of starch and had a negative rotation, but in all other cases a certain amount of starch went into solution.

Moreover, this method of correcting for the soluble proteins assumes that the same amount of protein would dissolve in cold, as in boiling, calcium chloride solution. A simple experiment showed that this is not true. Starch-free wheat gluten (0.350 gm.) gave the following rotation: hot solution, −.066°; cold solution, −.049°. This difference would give rise to an error of about 0.4% in the result for starch.

Similarly, when sucrose is dissolved in cold calcium chloride solution it has not the same rotatory power that it has when dissolved in hot calcium chloride solution. When treated with boiling calcium chloride solution sucrose is partially or wholly inverted. Its rotation after solution in cold aqueous calcium chloride is not far from normal, being affected only by the high salt concentration. Pure sucrose (5.00 gm.) gave the following rotation: hot solution, −1.512°; cold solution, +3.152°.

In several cases, zinc chloride was added to the solution obtained by treating wheat with hot aqueous calcium chloride in order to remove the proteins. There was a precipitate in each case but it was difficult to filter off, and the filtrates were not very clear for polarizing. It was also noted that the zinc chloride was hydrolyzing the starch rather rapidly, as shown by the following figures: rotation after 0 hours, +2.853°; 2 hours, +2.800°; 2½ hours, 2.787°. The use of zinc chloride was discontinued.

Other Experiments

A test was made of the revised method to determine whether hydrolysis of starch occurred. The final solution was polarized at successive intervals, with the following results: rotation after 0 hours, +2.812°; 2½ hours, +2.810°; 7½ hours, +2.801°; 21½ hours, 2.803°. The readings indicate that there is little if any hydrolysis in the very weakly acid solution.

Wheat starch was dispersed completely in boiling formamide. The solution gelled on cooling, however, and could not be filtered.

A solution of starch in aqueous calcium chloride (rotation, +5.392°) was poured into 2½ volumes of 95% alcohol. The precipitate of starch was filtered off on a Gooch, redissolved in aqueous calcium chloride and brought to the original volume. Its rotation was +5.160°. Evidently the precipitation by alcohol was incomplete.

A solution of starch in aqueous calcium chloride (rotation, +3.506°) was treated with excess of iodine plus potassium iodide and allowed to stand overnight for complete precipitation. The starch iodide was filtered off and the filtrate decolorized with sodium thiosulphate. After it was made up to double the original volume, its rotation was +0.167°, equivalent to +0.334° for the original solution. It gave no color with further additions of iodine.

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CHEMICAL STUDIES ON APPALACHIAN UPLAND PODSOL SOILS

II. ORGANIC MATTER-ACIDITY RELATIONS[1]

By H. J. Atkinson[2] and R. R. McKibbin[3]

Abstract

When leached with distilled water, the raw humus of the A_1 horizon of Quebec virgin podsols gave percolates of gradually increasing pH values over periods of 14 weeks, beginning in two instances at 4.3 and rising to 5.4, and in a third at 3.8, rising to 4.8. When concentrated by evaporation to from one-tenth to one-twentieth of the original volume the percolates retained 83.5 to 99% of their total acidity. The concentrates were fractionated by dialysis through cellophane. From 46 to 91% of the acidity dialyzed. In the dialyzates from five soils, 47.5 to 56.4% of whose acidity dialyzed, total sulphuric acid was found in amounts varying from 30 to 52% of the total acidity. The free (alcohol-soluble) sulphuric acid amounted to 9.5 to 23% of the total acidity of the percolate. Phosphoric acid was present in mere traces in some samples, but in one instance the total phosphoric acid content was sufficient to account for 28% of the total acidity, if it was present in the free state.

Introduction

The characteristic ash-like A_2 horizon of a virgin podsol soil results from severe leaching of the horizon by an acid-reacting solution which percolates from the surface organic matter or "raw humus" A_1 horizon. Practically no information on the composition or nature of this acid solution is available. Glinka (1) stated that "the investigation of the humic material in the podsols shows for horizon A_1 the presence of substances which are known under the names of humic and crenic acids . . . . If the solution reacts acid, the humic acid cannot be extensively removed, for it is soluble only in alkaline solutions. The crenic acid, on the other hand, can be removed to considerable depths by water." Apparently Glinka believed that "crenic acid" is chiefly responsible for the leaching in podsol soils, but with reference to this acid, Neustruev (4) remarked that "no one has proved the presence of crenic acid, and in general its existence and properties are thus far hypothetical." A somewhat similar statement was made by Morgan and Lunt (3) to the effect that "Perhaps we have ceased to believe in hypothetical crenic and apocrenic acids, but we cannot gainsay the action of forest organic matter of strongly acid reaction in the podsolizing process."

The extremely acid nature of the horizon of organic matter accumulation in the podsols is a generally accepted fact. The close relation existing between the organic matter and the acidity of long-cultivated podsols in the Appalachian uplands of Quebec has been pointed out by Shaw and McKibbin (6). They plotted the lime requirement in pounds of calcium oxide per acre, representing the acidity, against the percentage of organic carbon, representing the organic matter, and they found that a direct relation was indicated.

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[2] *Research Assistant, Macdonald College.*
[3] *Assistant Professor of Agricultural Chemistry, Macdonald College.*

They showed also that a similar relation exists when the replaceable hydrogen (expressed in milliequivalents) in the base-exchange complex is plotted against the percentage of organic carbon. In undertaking a detailed investigation of the problem of acidity in these soils, it seemed logical to begin the study with the organic matter. In the virgin podsol, it is quite easy to separate the horizon of organic matter accumulation from the neighboring horizons and to obtain a material which is made up almost entirely of the annual deposits of leaves, needles, twigs and other forest litter. This is in a semi-decomposed fibrous condition and has an extremely acid reaction. In the case of every sample subjected to investigation in this work, care was taken to ascertain that a well developed leached layer was present beneath the raw humus layer used. The surface A_0 horizon of undecomposed material was removed, as was any trace of the A_2 leached horizon. The procedure followed in this investigation was worked out step by step, as no satisfactory method was available at the start. Each successive step was carried out in such a way that as little change as possible would be brought about in the original constituents.

Experimental and Discussion

Treatment of Samples

The organic matter of the A_1 horizon, or "raw humus," was first passed through a screen ($\frac{1}{4}$ in. mesh), and roots and other undecomposed material were removed as completely as possible. The screened material was then put into glass percolators, the bottom openings of which were covered with fine gravel which had been previously digested with hydrochloric acid and sodium hydroxide and then carefully washed with distilled water. The gravel served as a support for the soil and did not interfere with the percolation of liquid. Distilled water was added at the surface, and the liquid that came out at the bottom was collected for examination. This method is similar to that employed by Parker (5) in his investigation of the soil solution of cultivated soils. The liquid obtained in this way from the "raw humus" of virgin podsol soils should correspond closely to the material active in bringing about podsolization. In the field, rain water passes through the organic matter layer and carries with it those substances which act on the strong bases and sesquioxides of the underlying horizons, dissolving and leaching them to lower levels, leaving the bleached podsol horizon. The addition of distilled water in the laboratory is analogous to the addition of rain water in the field. Free drainage is permitted in the percolator because the sample is supported only on loose fine gravel. Free drainage can also take place in the field. In the percolator, the raw humus is saturated with water before any liquid comes out at the bottom. Such conditions also exist in the field, especially in the spring after the winter snows have melted, and perhaps also at intervals throughout the summer and autumn after heavy rainstorms.

Distilled-water Percolate

The distilled-water percolate obtained as outlined above is a clear, dilute, somewhat colored, strongly acid solution. Its acid nature is easily shown by

determining the hydrogen ion concentration. Since the solution is colored, this cannot be done colorimetrically. In this work, it has been done electrometrically, using a Leeds and Northrup type K potentiometer, a saturated potassium chloride-calomel half-cell and bubbling hydrogen electrode. Successive percolates taken at intervals of several days over a period of some weeks continued to be strongly acid.

In Table I are presented data obtained with percolates from three different soils. The percolates were taken at intervals of two weeks for a period of 14

TABLE I

DISTILLED-WATER PERCOLATES TAKEN AT INTERVALS OF TWO WEEKS FROM 500 GM. OF RAW HUMUS (WEIGHED AIR-DRY)

	No. of weeks after first addition of distilled water						
	2	4	6	8	10	12	14
Soil No. 1							
pH	4.26	–	4.85	5.21	5.29	5.39	5.36
Total solids							
On evaporation, %	0.31	0.27	–	0.16	0.14	0.15	0.13
On ignition to 650° C., %	0.022	0.029	0.019	0.017	0.014	0.017	0.010
Soil No. 2							
pH	4.32	4.46	4.90	–	5.14	5.63	5.35
Total solids							
On evaporation, %	0.20	0.21	–	0.15	0.13	0.13	0.11
On ignition to 650° C., %	0.017	0.028	0.018	0.022	0.016	0.014	0.010
Soil No. 3							
pH	3.77	3.96	4.29	4.52	4.57	4.75	4.82
Total solids							
On evaporation, %	0.23	0.16	–	0.14	0.14	0.15	0.16
On ignition to 650° C., %	0.027	0.022	0.019	0.020	0.010	0.014	0.015

weeks. There was 500 gm. of air-dried soil in each percolator, and 500-cc. percolates were collected. The origins of the soils are as follows:—

Soil No. 1—Brome County, vegetative cover—mixed, birch predominating.

Soil No. 2—Compton County, vegetative cover—hard maples.

Soil No. 3—Sherbrooke County, vegetative cover—evergreen, chiefly pine.

The results show that, even after a period of 14 weeks, the percolate continued to be extremely acid, although there was a gradual falling off in the intensity of the acidity as indicated by a gradually increasing pH value. The contention that the organic matter is a potential source of acidity in the podsol soils is strengthened by these results.

The figures in the table also give an indication of a gradual decrease in the content of total solids of successive percolates, both on evaporation and on ignition to 650° C. The greater part (about 90%) of the total solids in these percolates was organic in nature and was driven off on heating to 650° C.

Concentration of Percolates

The dilute nature of the percolates made it necessary to concentrate the solutions to obtain convenient amounts with which to work. It was a very easy matter to obtain a large volume of percolate from a given soil by setting up a battery of percolators and displacing the solution from each. The percolates were evaporated in a distilling flask under reduced pressure at temperatures below 30° C. Tests showed that the liquid distilling off was neutral in reaction and that the acidity became concentrated in the liquid remaining undistilled. This method of concentration has a number of disadvantages, especially when large volumes of liquid are to be concentrated. Further investigation showed that it is not necessary to carry out concentration at such low temperatures. When the percolates were allowed to evaporate in porcelain basins on a steam bath until reduced to from one-tenth to one-twentieth of the original volume, a very high percentage of the acidity was recovered in the concentrate. This was shown by titrating aliquot samples of the percolate both before and after concentration. The hydrogen ion concentration of the sample was determined electrometrically and standard *N*/10 sodium hydroxide solution was added gradually, with stirring, from a microburette until the reading on the potentiometer corresponded to that for pH 7. From the titration values so obtained, the amount of sodium hydroxide necessary to neutralize the whole of the acidity present both before and after concentration was determined, and the percentage recovery of acidity calculated. The results obtained with a number of percolates from different samples of raw humus are presented in Table II. They indicate the results that may be expected from this method of concentration.

TABLE II

RELATION BETWEEN THE ACIDITY OF ORIGINAL PERCOLATES AND THAT OF THEIR CONCENTRATES

Soil No.	Volume of distilled water percolate, cc.	Volume of concentrate after evaporation on steam bath, cc.	Titration values, cc. 0.1 *N* NaOH		% of original acidity in concentrate
			Original percolate	Concentrate	
4	5,850	600	68.0	64.2	94.4
5	17,900	940	683.5	587.4	86.0
6	15,690	780	147.4	123.0	83.5
7	10,000	660	174.2	160.6	92.2
8	8,000	875	271.0	268.7	99.1
9	6,000	650	129.0	121.7	94.4

The origin of each soil is as follows:—

Soil 4—Compton Co., near Eaton Corners; vegetative cover—evergreen.

Soil 5—Jacques Cartier Co., near Ste. Anne de Bellevue; vegetative cover —evergreen.

Soil 6—Compton Co., near Sawyerville, on Scott farm; vegetative cover—evergreen.

Soil 7—Compton Co., near Sawyerville, on Bulwer road; vegetative cover—evergreen.

Soil 8—From same location as Soil 4, but sample taken one year later.

Soil 9—Compton Co., near Sawyerville, on Taylor farm; vegetative cover—evergreen.

The solutions resulting from the concentration of the percolates were very dark brown, colloidal and opaque. Flocculent precipitates of the colloidal materials could be obtained by the addition of solutions of heavy metal salts, such as lead nitrate, copper sulphate and silver nitrate. A flocculent precipitate was also obtained when the concentrated solution was poured into a large excess of 95% ethyl alcohol.

Dialysis of Percolates

The next step in the examination of the concentrated percolate was dialysis. The purpose of this step was to separate the acids in true solution from those in colloidal solution or associated with colloidal particles. For this purpose cellophane was used and found to be a satisfactory dialyzing membrane. A sheet about 20 in. square can be formed into a sac which will easily contain 600 to 700 cc. This was done by folding the cellophane over the bottom of a large beaker, passing a cord around it, removing the beaker and tightening the cord. The sac was suspended in the centre of a large crystallizing basin or inverted bell jar, and the material to be dialyzed was introduced into it by means of a funnel. The sac was then surrounded with distilled water.

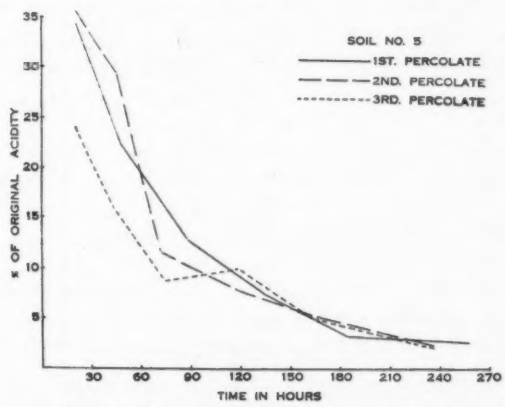

FIG. 1. *Decrease in dialyzable acidity during dialysis for the three percolates from Soil No. 5.*

The water in the dish was changed about once a day. Successive dialyzates over a period of several days showed a gradually decreasing concentration of acidity. Here, as in the previous work, the acidity was determined by electrometric titration of aliquot samples. The dialyzates were colored, owing to the fact that some

organic matter dialyzed, thus precluding the possibility of using colorimetric titration methods. After a period of seven or eight days, the acidity of the dialyzates usually fell off to a very small amount, about 2% or 3% of the original acidity. There was, however, still remaining within the sac a large portion of the acidity which could also be determined by electrometric titration. Thus it was possible to separate the acidity of each percolate into a dialyzable and a non-dialyzable fraction. Furthermore, when the total acidity of the successive dialyzates was added to that of the non-dialyzable fraction, a recovery of usually about 90% of the total acidity was obtained, which may be within the limits of accuracy of the method. Actual figures obtained with two soil samples are given in Tables III and IV, and the results are shown graphically in Figs. 1 and 2.

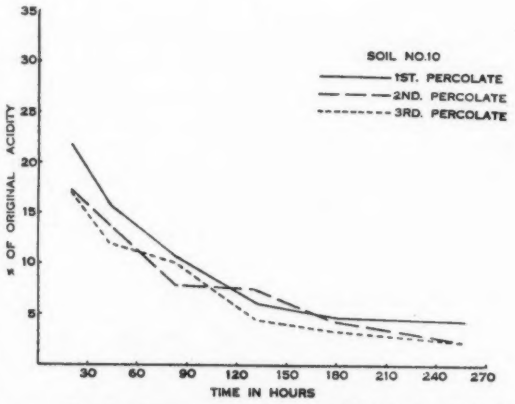

FIG. 2. *Decrease in dialyzable acidity during dialysis for the three percolates from Soil No. 10.*

TABLE III

DATA SHOWING DECREASE OF ACIDITY IN SUCCESSIVE DIALYZATES, SOIL NO. 5. THREE PERCOLATES TAKEN AT INTERVALS OF THREE DAYS

Dialyzate No.	First percolate		Second percolate		Third percolate	
	Time of dialysis, hr.	% of original acidity	Time of dialysis, hr.	% of original acidity	Time of dialysis, hr.	% of original acidity
1	21	34.1	21	35.3	20	24.1
2	28	22.1	24	29.4	24	15.9
3	39	12.9	27	11.6	31	8.8
4	48	7.4	46	7.8	44	10.0
5	49	3.1	48	5.0	46	4.8
6	74	2.5	71	2.1	72	2.0
Total dialyzed acidity		82.1		91.2		65.6
Acidity remaining within sac		12.1		8.7		22.6
Total acidity accounted for		94.2		99.9		88.2

TABLE IV

DATA SHOWING DECREASE OF ACIDITY IN SUCCESSIVE DIALYZATES, SOIL NO. 10.* THREE PERCOLATES TAKEN AT INTERVALS OF THREE DAYS

Dialyzate No.	First percolate		Second percolate		Third percolate	
	Time of dialysis, hr.	% of original acidity	Time of dialysis, hr.	% of original acidity	Time of dialysis, hr.	% of original acidity
1	20	21.8	20	17.4	20	17.1
2	24	15.7	24	13.8	24	12.0
3	39	10.7	39	8.0	39	10.1
4	49	6.1	48	7.5	49	4.5
5	48	4.7	48	4.3	47	3.3
6	77	4.3	78	2.3	78	2.2
Total dialyzed acidity		63.3		53.3		49.2
Acidity remaining within sac		27.1		35.1		37.8
Total acidity accounted for		90.4		88.4		87.0

**Soil No. 10—Compton Co., near Sawyerville, on Halliday farm; vegetative cover—largely evergreen.*

Sulphuric and Phosphoric Acids in Dialyzates

Having obtained a definite separation of the acidity, the next step was an investigation of the chemical constitution of the fractions. Little definite information has been obtained on the structure of the non-dialyzable portion. Oxidation with dilute alkaline potassium permanganate solution, even at temperatures below 20° C., yielded only oxalic acid. Other oxidizing agents destroyed its colloidal nature, but so far no substances have been identified which would give any indication of the original structure.

In the dialyzable fraction, the presence of sulphate and phosphate ions has been shown. This indicates that sulphuric acid and phosphoric acid or their acid salts may be responsible for a large part of the dialyzable acidity. Furthermore, the presence of free sulphuric acid has been shown. During recent years, there has been a number of references in the literature to the importance of sulphuric acid in the weathering of rocks. Organic materials are regarded as the source of much of the sulphuric acid present in soils, it being formed as the result of complete oxidation of the sulphur-containing protein substances of plants. One of the most recent papers on this subject is by Heimath (2), who gives a good review of the literature and attributes to Blanck an important part in the investigation of the role of sulphuric acid in soils. Heimath studied the acid organic matter of a number of soils, using a method of electro-ultrafiltration to separate the ions, and showed that the sulphate ion was present in large amounts. He concludes that the free acid must be present to account for the very low pH values obtained.

The distilled-water percolates of a number of soils examined in this work had pH values ranging from 5.5 to 2.9. The most acidic of these, after concentration, showed a value of pH 2.5 for the first dialyzate. Such low

pH values can be caused only by some strong, highly dissociated acid. Hydrochloric acid and nitric acid are never present in sufficient amounts to influence the hydrogen ion concentration to this extent, according to the writers' analyses. There remains the possibility that sulphuric acid and phosphoric acid are responsible. In this investigation, the successive dialyzates of a number of samples have been analyzed for sulphate and phosphate content. The method has been to take the complete dialyzate or a definite fraction of it to dryness in the presence of magnesium nitrate solution as a flux, ignite to about 550° C. to remove all volatile material, take up in concentrated hydrochloric acid, precipitate sulphate as barium sulphate and phosphate as ammonium phosphomolybdate. A blank determination was run on all the reagents used, and the value of the blank was deducted. Sulphate was found in all samples examined, but the presence of phosphate was not so general. It has been found in considerable amount in most cases, but in some it was present in very small quantities only. In Table V are presented the

TABLE V

SULPHATE AND PHOSPHATE CONTENTS OF DIALYZATES, EXPRESSED IN TERMS OF THE ACIDITY OF THE SAMPLES

Soil No.	Percolate No.	% dialyzable acidity accounted for as H_2SO_4	% dialyzable acidity accounted for as H_3PO_4	% total acidity that is dialyzable	% total acidity accounted for as H_2SO_4	% total acidity accounted for as H_3PO_4
5	1st	67.5	1.0	87.1	58.8	0.9
	2nd	23.1	0.4	91.3	21.1	0.3
	3rd	21.1	1.3	74.4	15.7	1.0
6	6th	61.3	17.3	54.6	33.5	9.5
	7th	60.0	20.4	46.4	27.9	9.5
10	1st	90.1	7.2	70.0	63.0	5.0
	2nd	26.9	8.5	60.3	16.2	5.1
	3rd	24.5	15.0	56.6	13.9	8.5
11	1st	140.5	10.5	75.7	106.4	7.9
	2nd	49.9	46.2	75.2	37.5	34.7
12	2nd	79.0	Trace	62.6	49.5	—
	3rd	128.4	Trace	52.2	67.0	—

results obtained for sulphate and phosphate content of the dialyzates of a number of percolates from the "raw humus" horizon of typical virgin podsols. The origin of some of these soils has been given above. Of the others, Soil No. 11 is from Brome Co., two miles from Knowlton, cover—mixed evergreen and deciduous; Soil No. 12 is from Shefford Co., near Stukely, cover—largely evergreen with some deciduous. The total sulphate content has been calculated as sulphuric acid and this, expressed as percentage of the dialyzable acidity, is given in the third column. Similarly, the total phosphate

content, calculated as phosphoric acid, is expressed as percentage of the dialyzable acidity in the fourth column. The fifth column shows, for each sample, the percentage of the total acidity that is dialyzable. From this and the results in the third column, it is possible to calculate the percentage of the total acidity that may be accounted for as sulphuric acid when the total sulphate content is calculated as sulphuric acid. The result is shown in the sixth column. Similarly, from the results in the fourth and fifth columns, it is possible to calculate the percentage of the total acidity that can be accounted for as phosphoric acid, when the total phosphate content is calculated as phosphoric acid. The results so obtained are given in the seventh column.

From the results presented it is seen that the sulphate and phosphate contents of these soils are sufficient to account for a very large portion of the water-soluble acidity, if the acids are present in the free state. One very significant point is that these acids arise entirely in the horizon of organic matter accumulation. They are probably not present entirely in the free state, but partly as neutral or acid salts, owing to the effect of cations of the mineralized part of the organic matter. This conclusion is indicated by the results from Soil No. 11, first percolate, where more than sufficient sulphate is present to account for the entire acidity if it were present entirely as free sulphuric acid. Nevertheless, a considerable amount of these acids must be present in the free state, in order to account for the low pH values of the percolates.

Free Sulphuric Acid

In order to determine what proportion of the sulphate content was present as free sulphuric acid, the solution was concentrated to a small volume (about 50 cc.) and poured into a large volume (about 450 cc.) of 95% ethyl alcohol. Practically all the sulphates were thus precipitated, leaving the free sulphuric acid in solution. Raw humus samples from five virgin podsols, well distributed over the Appalachian upland region of Quebec province, were examined in this way for free sulphuric acid, and these five are the only ones thus far studied in this connection. Three percolates from each soil were taken at intervals of three days, concentrated and dialyzed for 60 hr. The water was changed at 16, 20 and 24 hr. Thus dialysis was not continued until all the dialyzable acidity was removed. Previous work showed, however, that by far the greater part of the acidity is removed in the first three dialyzates, and it is believed that the results obtained in this manner give a good indication of the amount of free sulphuric acid present. Each dialyzate was divided into two equal portions. One was analyzed for total sulphate and the other for free sulphuric acid according to the method outlined above. Total phosphate content was also determined in the fraction that was analyzed for total sulphate.

The results obtained are presented in Table VI and are expressed as percentage of dialyzable and total acidity as was done in Table V. Here, however, the results obtained for the three dialyzates from each of the three per-

TABLE VI

PHOSPHATE AND TOTAL AND ALCOHOL-SOLUBLE SULPHATE CONTENTS OF DIALYZATES, EXPRESSED IN TERMS OF THE ACIDITY OF THE SAMPLES

Soil No.	% dialyzable acidity accounted for as H_2SO_4 from total sulphate	% dialyzable acidity accounted for as H_3PO_4	% total acidity that is dialyzable	% total acidity accounted for as H_2SO_4 from total sulphate	% total acidity accounted for as H_3PO_4	% dialyzable acidity accounted for as H_2SO_4 from alc.-sol. sulphate	% total acidity accounted for as H_2SO_4 from alc.-sol. sulphate
7	69.8	Trace	47.5	33.1	—	20.1	9.5
8	55.3	Trace	55.1	30.5	—	26.4	14.6
9	92.8	Trace	56.4	52.4	—	40.6	22.9
13	78.8	5.7	53.8	42.4	3.1	19.7	10.6
14	57.4	54.2	52.1	29.9	28.2	32.6	17.0

colates of each soil have been added together in order to get one set of figures for each soil examined. The locations of soils Nos. 7, 8 and 9 have been given previously. Soil No. 13 was from Kamouraska Co., about five miles south of St. Onésime. The cover was largely evergreen but contained some deciduous trees. Soil No. 14 was from Montmagny Co., one mile west of St. Paul. The cover was about the same as for soil No. 13. Columns 2 to 6 of Table VI correspond to Columns 3 to 7 of Table V. In the seventh column of Table VI, the sulphate content of the fraction not precipitated on being poured into excess of alcohol has been calculated as sulphuric acid and expressed as percentage of dialyzable acidity. This has been converted into percentage of total acidity of the samples in the eighth column.

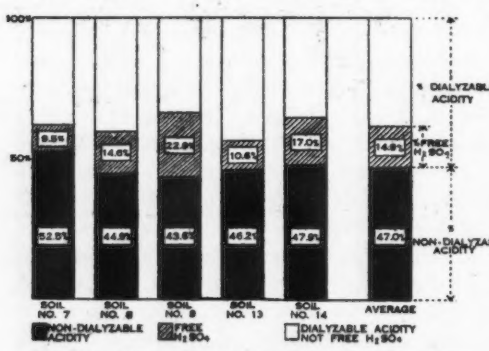

FIG. 3. *The relative amounts of dialyzable and non-dialyzable acidity, showing also the amount of free sulphuric acid in five representative Appalachian upland podsol soils of Quebec.*

In Fig. 3, the results for each of these five soils, together with the average results, are expressed graphically so as to show the relative amounts of acidity present as non-dialyzable acidity (in solid black) and as dialyzable acidity. The dialyzable fraction is subdivided to give the amount of free sulphuric acid determined (shaded). The remainder of the readily dialyzable fraction is represented by the unshaded part. This particular fraction of the acidity is now being studied in this laboratory.

As in Table V, the results here show the presence of phosphoric acid or phosphates in considerable amount in some cases, but only in very small

amounts in others. Sulphuric acid was, however, present in appreciable quantities in all cases, and a large fraction of it existed in the free state. In the five soils examined, sufficient free sulphuric acid was present to account for from 9.5% to 22.9% of the total acidity of the distilled-water percolates. Incidentally, it may be mentioned that the cultivated soil within 100 yards of the place where soil No. 9 (which showed the highest percentage of free sulphuric acid (22.9%)) was obtained, is one of the poorest soils in this region.

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FLOW FORMS IN A CHANNEL OF SMALL EXPONENTIAL DIVERGENCE[1]

By G. N. Patterson[2]

Abstract

The motion of air through a channel of small exponential divergence has been investigated experimentally. A flow form derived by Blasius from theoretical considerations has been shown to exist in the range $25 \leqslant R \leqslant 36$ for the Reynolds number. The dependence of the general flow form on the initial velocity distribution where the divergence begins has been studied. It has been found that when this initial velocity distribution is parabolic, indicating a laminar motion in the throat of the channel, the flow form is symmetrical. Further investigations have shown that when the initial velocity distribution indicates that the motion near the walls in the throat of the channel lies in the transitional region between a laminar and a turbulent flow, then the flow form is unsymmetrical. Empirical equations have been obtained which give (1) the initial velocity distribution in the transitional region at $R=75.1$, and (2) the motion near the walls where the divergence begins for Reynolds numbers lying in the range $0 \leqslant R \leqslant 75.1$.

Introduction

The problem of the flow of a viscous incompressible fluid between two walls which diverge symmetrically according to an exponential relation has been treated theoretically by Blasius (1). The flow was regarded as a steady two-dimensional motion and the walls were considered to diverge very gradually in a manner given by the equation $z=a+be^{\epsilon x}$, where ϵ is small. In order to complete the necessary boundary conditions, some form of initial velocity distribution at $x=0$ was required.

Blasius assumed a parabolic distribution and showed that the divergence of the channel developed a reversed pressure gradient which opposed the motion of the fluid, causing it to break away symmetrically from both walls of the channel. Equations of velocity distribution were obtained which showed the existence of a reversed flow in the neighborhood of the walls. The work of Blasius on this problem also indicated that the flow form between the diverging walls depended upon the initial velocity distribution at $x=0$ in the throat of the channel.

The present experimental work was undertaken (1) to verify the existence of the Blasius flow form and to determine the range of the Reynolds number for which it will be found, and (2) to study the relation between subsequent changes of the flow form and the corresponding variation of the initial velocity distribution at $x=0$.

Experimental Arrangement

Air was chosen as the medium to be investigated. A modification of the photographic method of Nisi and Porter (3) was adopted. A steady motion through the channel was maintained by a large tank from which water

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Contribution from the Physics Laboratory, University of Toronto, Toronto, Ontario, Canada.

[2] *This work was carried out while the author held a Research Fellowship in the Graduate School of the University of Toronto.*

siphoned at a constant rate. The flow was made visible by magnesium oxide particles. The region under investigation was illuminated by a flat sheet of light in the horizontal plane and the motions of the particle indicators were recorded on the film of a camera. From the film the length of the particle tracks could be determined, and, using the measured time of exposure, the velocity distribution could then be found.

The method of obtaining a steady flow is illustrated in Fig. 1. The air and magnesium oxide particles are mixed in the box, *A*, and then allowed

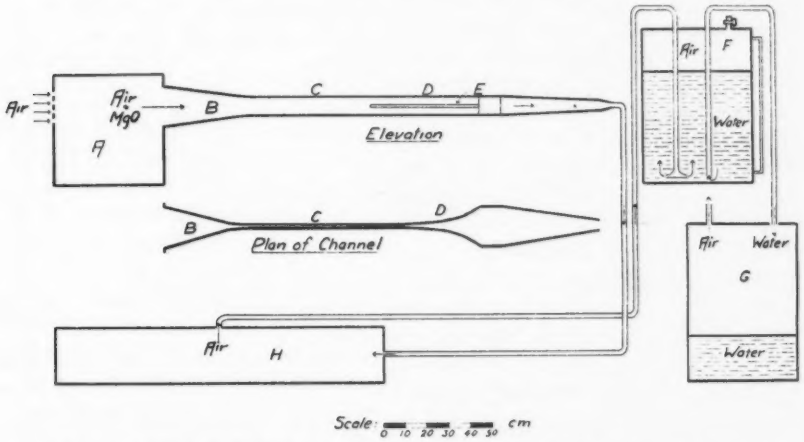

FIG. 1. *Diagram of the general arrangement showing the method of obtaining a steady flow, and the dimensions of the channel.*

to settle so that air currents and large particles of the powder could be eliminated. Water in the tank, *F*, is permitted to siphon into the tank, *G*, at a constant rate. The air bubbles into the tank, *F*, through an inlet which is placed at the same level as the outlet for the water. The velocity can be varied by opening or closing a rubber section of the siphoning tube with a clamp. The mixture of air and particles then passes through the reducing section, *B*, designed to stabilize the flow, into the narrow channel, *C*. Through a cellophane window, *E*, in the divergent section, *D*, a flat sheet of light is passed which illuminates the central horizontal plane of the channel. The particles are then clearly visible and can be photographed through a glass window fitted on top of the divergent section, *D*. The mixture continues through a second reducing section into a long pipe and thence into the large reservoir, *H*.

The end of the pipe through which the air issues into the tank, *F*, is fitted with four small jets which allow the air to escape into the water in small bubbles which form so rapidly that any tendency of the water to cover the mouth of the jet after the formation of each bubble is checked. This arrangement and the large tank, *H*, with its long connecting pipes, serve to eliminate

any pressure oscillations due to the formation of bubbles in the tank, *F*. It will be seen that the experiment is arranged so that the powder does not enter the room. Compressed air is used to force the water from the tank, *G*, back into the tank, *F*. The channel is made of wood with the exception of the vertical walls of the divergent section which are made of brass smoothly fitted into place. Over the inside surface of the brass is stretched a thin sheet of cellophane.

It was found that the best photographs of particle tracks were obtained when magnesium oxide powder was used, owing to its lightness and its high reflecting power. If the particles were not too large they remained suspended in still air and the rate at which they settled out was negligible compared with the velocities used in the channel. Fig. 2 shows the method of obtaining a good mixture of air and particles. Compressed air enters the tube, *A*, and mixes with the powder in the container, *B*. The mixture then passes through the tube, *C*, into the chamber, *D*, where it encounters a series of baffles, *E*, which complete the mixing process. It then enters the box, *A*, shown in Fig. 1.

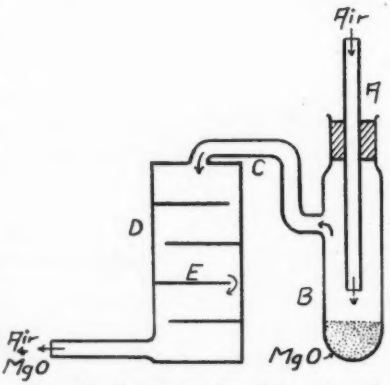

FIG. 2. *Apparatus for mixing air and magnesium oxide powder.*

In the optical system the source of light is an electric arc capable of carrying 30 to 60 amp. The light is collected by a large condenser and transmitted through a water cell. The rays then converge on a narrow slit. The image of the slit is projected through the cellophane windows (*E*, Fig. 1) of the channel by a projection lens. This lens is achromatic and possesses an aperture of 2 in. and a focal length of 2 in. The reflected light from the particles thus illuminated is collected by a photographic lens (2.5 cm. aperture, 5 cm. focal length), and is then projected on the high speed superpanchromatic film of a camera. The camera is placed above the illuminated plane with its axis vertical.

Experimental Technique

Before commencing to take photographs the flow forms corresponding to increases of the mean velocity, $\bar{u}$, from rest up to 22.5 cm. per sec. were observed. Values of $\bar{u}$ at which type flow forms were observed were recorded. Photographs of these type flow forms were taken and then, at corresponding values of $\bar{u}$, photographs were made of particle tracks in the throat of the channel (*i.e.*, in the region of $x=0$) from which the initial velocity distribution for each flow form was determined. No observations were made when the velocity was higher than the mean velocity of 22.5 cm. per sec., since at greater speeds the particle tracks on the film were not sufficiently clear for accurate measurement.

These observations were made for a narrow channel of very gradual divergence. The straight walls (see Fig. 1) bore a ratio of height to width of 16. This ratio decreased as the channel diverged and it was limited by the short distance from the camera lens to the central horizontal plane of the channel which was necessary for obtaining clear photographs of the particles. The walls of the channel were curved according to the relation

$$\pm z = 0.25e^{0.1x} , \tag{1}$$

where x is the distance along the axis of the channel and $\pm z$ are the distances from the axis to the respective walls. The divergence began at $x=0$, and for $x<0$ the walls were straight and parallel, being separated by a distance of 0.5 cm.

The measurements of velocity distribution required the accurate determination of the time of exposure of the camera and the length of the particle tracks on the photographic film. Shutter speed calibrations were made accurately to 0.0001 sec. by a stroboscopic method immediately after the experiments were complete. A traveling microscope mounted on a cast iron base was used for the measurement of the particle tracks (to 0.001 cm.). The negative was placed between two thin pieces of glass and fitted securely into a holder which was firmly bolted to the cast iron base. The microscope was then accurately aligned. Since the level of the base was adjustable the alignment could be carried out with a level bubble. The determination of the position of each wall on the photographs was aided by observing the position of small particles adhering to the wall. Particles which were out of focus, and therefore not in the plane being measured, were easily picked out owing to the very short depth of focus of the camera lens.

Corrections to the measurements were made whenever they were appreciable. The sources of error were: (1) the fact that the particles could not be regarded as point particles (diameter of the order of 10^{-3} cm.); (2) the coarse grain effects on the negative due to the use of a high speed film; (3) the focal plane shutter of the camera (hardly appreciable even at the highest velocities).

All observations and measurements were confined to the central horizontal plane of the channel. The mean velocity, $\bar{u}$, is that corresponding to this plane and is obtained from the photographs. The photographs of the flow forms are all time exposures of 5 to 10 sec., whereas the photographs of the particle distributions were made at 1/20, 1/30, 1/40, 1/60 sec. All observations were made at a constant room temperature of 23° C.

Results

Photographs of the observed types of flow forms are shown in Figs. 3–6. There are two distinct types: (1) a symmetrical flow (Figs. 3 and 4); (2) an unsymmetrical flow (Figs. 5 and 6). The photographs represent a length of about 25 cm. Since the direction of flow is from left to right it will be seen that reversed flow regions exist which characterize each flow form. These

regions are easily distinguished from the main flow since the density of the particle distribution in the return flow areas is much less than that of the main flow.

At a mean velocity of 5.6 cm. per sec. the flow form is three-dimensional and symmetrical (Fig. 3). After passing the point where the divergence begins ($x=0$) the main flow develops a downward component of velocity and disappears from the central horizontal plane under observation. This is the white area issuing from the throat of the channel and terminating near the centre of the photograph. In the wide section of the channel two reversed flow regions exist. As the particles enter the field of view they are seen to be moving along the walls toward the narrowest part of the channel. Upon nearing the region where the main flow disappears the particles move away from the walls toward the centre of the channel. This change in position with respect to the walls is accompanied by a rapid reversal of the direction of motion. The particles finally disappear from the field of view after a rapid transit along the centre of the channel.

When the mean velocity is increased to 10.3 cm. per sec. the flow form is two-dimensional and remains symmetrical (Fig. 4). The main flow now extends throughout the full length of the divergent section of the channel and separates the return flow regions. These regions occupy much smaller areas but the particles in them still move in the same characteristic way as described above. The flow leaves the wall at two corresponding points and moves in accordance with the predictions of Blasius.

When the mean velocity of 17.0 cm. per sec. is attained the flow form is unsymmetrical but remains two-dimensional (Fig. 5). The points at which the main flow leaves the walls no longer correspond and the flow deviates toward one wall. One of the return flow areas now becomes smaller and the other increases, though the particles in these regions still retain their characteristic motion.

At the maximum mean velocity of 22.5 cm. per sec. the flow is still two-dimensional but its unsymmetrical form is more pronounced (Fig. 6). The main flow breaks away from one wall and moves parallel to the other. A single reversed flow region exists in which the particles circulate as previously described.

The transitions of the flow forms from one to the other as the mean velocity is slowly increased through the range $0 \leqslant \bar{u} \leqslant 22.5$ cm. per sec. are gradual. At the lowest velocities the flow form, while preserving its symmetry and three-dimensional quality as shown in Fig. 3, changes continuously with the mean velocity. At first the bright area in Fig. 3 is small and extends outward only a short distance from the throat of the channel. With increase of mean velocity this area extends further out into the channel, while the reversed flow regions shift forward to a new position so that the general form remains the same. When a value $\bar{u}=7.5$ cm. per sec. is reached

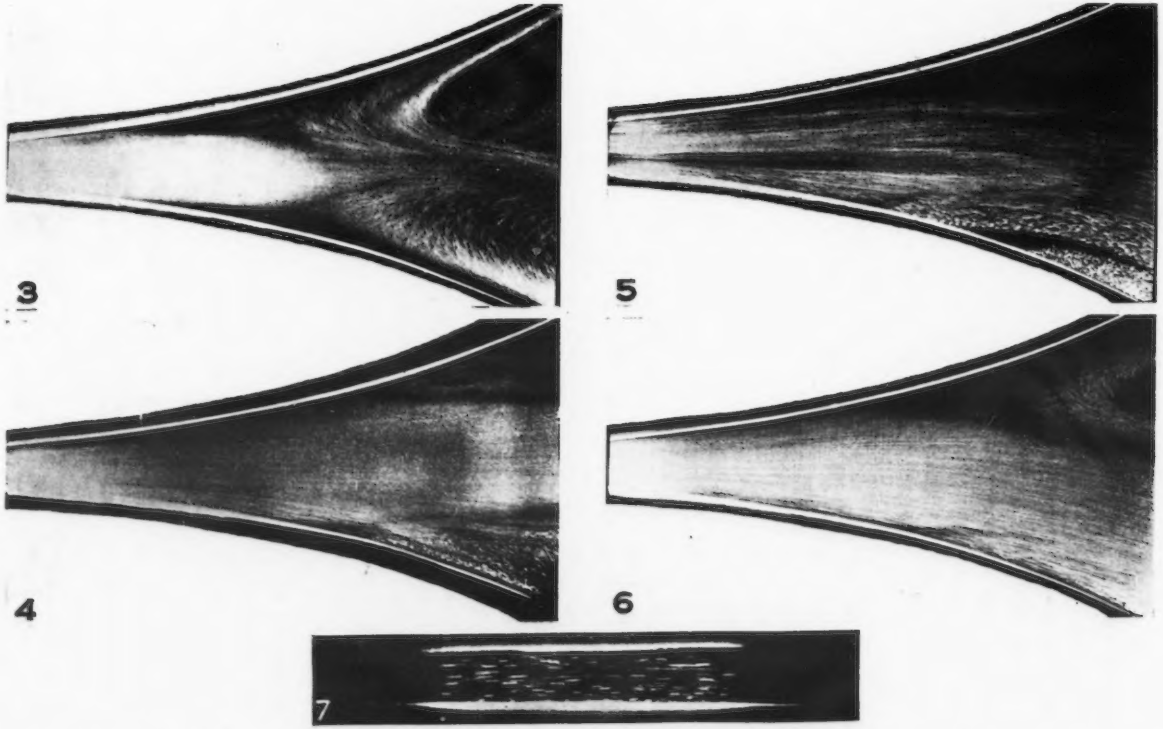

FIG. 3. *The three-dimensional symmetrical type of flow form at* $R=18.7$, $\hat{u}=5.6$ *cm. per sec.* FIG. 4. *The Blasius flow form at* $R=34.4$, $\hat{u}=10.3$ *cm. per sec.* FIG. 5. *The two-dimensional unsymmetrical type of flow form in the transitional stage at* $R=56.7$, $\hat{u}=17.0$ *cm. per sec.* FIG. 6. *The two-dimensional unsymmetrical type of flow at* $R=75.1$, $\hat{u}=22.5$ *cm. per sec. in its completed form at the end of the transition.* FIG. 7. *Particle tracks in the region* $x=0$ *which are typical of those from which measurements of the initial velocity distributions were made. Time of exposure, 0.0355 sec.*

PLATE I

the main flow extends across the field of view (Fig. 4) and the motion becomes two-dimensional. The flow form is now that described by Blasius. As the mean velocity continues to rise the Blasius flow persists for a time and then begins a very gradual transition at $\bar{u}=10.8$ cm. per sec. to that shown in Fig. 5. When observed in a large number of experimental trials it was found that the flow at this stage did not deviate toward the same wall in all cases, for during some of the trials the flow was observed to deviate to the left whereas in other cases the deviation was to the right. However, in any single trial, once the deviation toward one of the walls is begun, this deviation increases continuously as $\bar{u}$ is raised, until at $\bar{u}=21.0$ cm. per sec. the flow breaks away from one wall only. This flow continues with little change until the value $\bar{u}=22.5$ cm. per sec. is attained (Fig. 6).

Therefore two transitions occur: (1) the three-dimensional flow at low velocities becomes a two-dimensional flow at high velocities; (2) the symmetrical flow at low velocities changes to an unsymmetrical flow at high velocities.

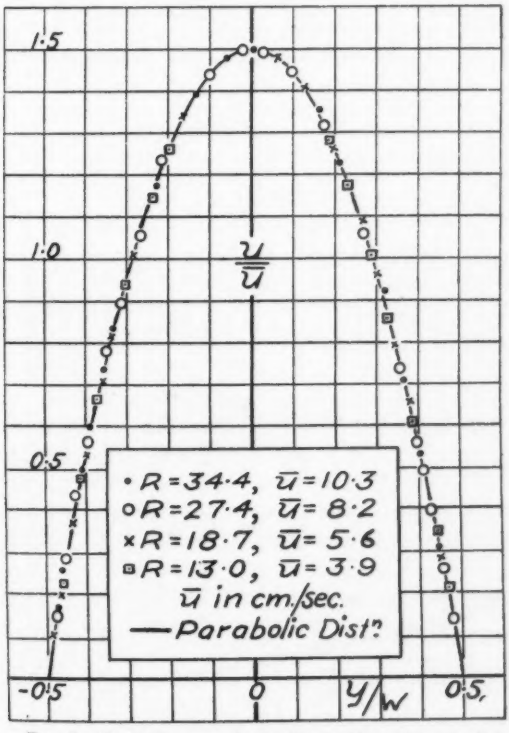

FIG. 8. *Experimental velocity distributions along the line $x=0$ at various values of $\bar{u}$ in the range $0<\bar{u}\leq 10.8$ cm. per sec. showing the existence of a laminar flow.*

A typical photograph of particle tracks from which measurements of the velocity distribution at $x=0$ were made is shown in Fig. 7. Representative points from the curves of velocity distribution obtained at $x=0$ for values of $\bar{u}$ of 3.9, 5.6, 8.2, and 10.3 cm. per sec. are plotted in Fig. 8. These values of $\bar{u}$ lie in the range ($0\leq\bar{u}\leq$ 10.8 cm. per sec.) in which the symmetrical type of flow form was observed (Figs. 3 and 4). Values of $u/\bar{u}$ are plotted against y/w, where u is the velocity at a point distant y from the axis of the channel, $\bar{u}$ is the mean velocity and w is the dis-

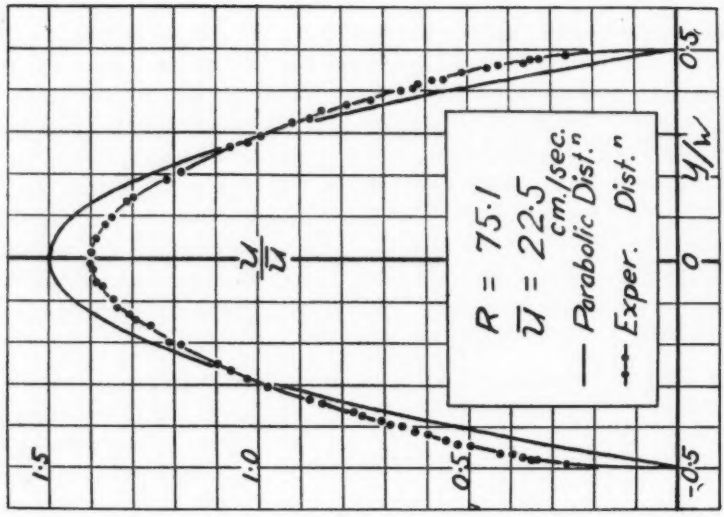

FIG. 10. *The experimental curve of velocity distribution at* $\bar{u} = 22.5$ *cm. per sec. for the line* $x = 0$ *indicating that the deviation from the laminar flow increases as* $\bar{u}$ *rises.*

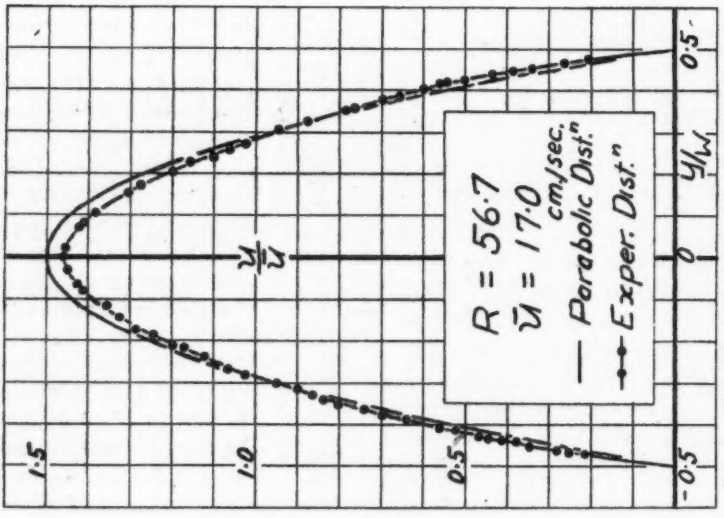

FIG. 9. *The experimental curve of velocity distribution at* $\bar{u} = 17.0$ *cm. per sec. for the line* $x = 0$ *showing the deviation of the flow from the laminar type.*

tance between the channel walls at $x=0$ (0.5 cm.). For comparison the parabolic distribution curve given by

$$\frac{u}{\bar{u}} = \frac{3}{2}\left[1-4\left(\frac{y}{w}\right)^2\right] \tag{2}$$

is also shown. It will be seen that the experimental points lie on the parabolic curve, and hence that the velocity distribution in the throat of the channel is parabolic in the range $0 \leqslant \bar{u} \leqslant 10.8$ cm. per sec.

The velocity distributions at $x=0$ for two values of $\bar{u}$ (corresponding to Figs. 5 and 6) in the range ($10.8 \leqslant \bar{u} \leqslant 22.5$ cm. per sec.) in which the unsymmetrical flow form was found are indicated in Figs. 9 and 10. A comparison with the parabola shows that the initial velocity distribution has now changed and the deviation from the parabolic form becomes greater as $\bar{u}$ increases. The curves in Figs. 8–10 are obtained at values of $\bar{u}$ corresponding to those of the flow forms shown in Figs. 3–6.

Discussion of Results

The experimental curve of velocity distribution in Fig. 10 is represented approximately by the empirical equation

$$\frac{u}{\bar{u}} = 1.40 - 8.48\left(\frac{y}{w}\right)^2 + 54.72\left(\frac{y}{w}\right)^4 - 172.4\left(\frac{y}{w}\right)^6 . \tag{3}$$

This is a particular form of the general expansion

$$\frac{u}{\bar{u}} = \Sigma a_n\left(\frac{y}{w}\right)^n , \qquad (n=0,2,4,\ldots) , \tag{4}$$

which, when evaluated to a sufficient number of terms, may be considered to give good agreement with the experimental velocity distributions shown in Figs. 9 and 10.

From Equation (3) it will be seen that, for small values of y/w the equation is approximately parabolic. Therefore, even at the highest velocities observed, the motion in the centre of the channel at $x=0$ is still essentially laminar. However, at large values of y/w the curve deviates quite appreciably from the parabolic form. The flow in those regions near the walls in the throat of the channel is no longer laminar. The increase of mean velocity to 22.5 cm. per sec. has therefore brought little change in conditions near the axis of the channel at $x=0$, but it has produced a non-laminar flow in the neighborhood of the walls at $x=0$.

The variation of velocity with the distance from the wall (4) as obtained from Figs. 8–10 in the range $0.4 \leqslant y/w \leqslant 0.5$ may be given in the form

$$\frac{u}{\bar{u}} = k\left(\frac{h-y}{w}\right)^n , \tag{5}$$

where $h=1/2w$. For a laminar flow $n=1$ and the conditions near the walls as shown in Fig. 8 are given by

$$\frac{u}{\bar{u}} = 5.70\left(\frac{h-y}{w}\right) . \tag{6}$$

The non-laminar distribution at the walls indicated in Fig. 9 may be written

$$\frac{u}{\bar{u}} = 4.00\left(\frac{h-y}{w}\right)^{0.302} . \tag{7}$$

Finally for $\bar{u}=22.5$ cm. per sec. (Fig. 10) the velocity varies with the distance from the wall in a manner given by

$$\frac{u}{\bar{u}} = 1.77\left(\frac{h-y}{w}\right)^{0.423} . \tag{8}$$

These values of n are plotted against the corresponding values of the Reynolds number*, R, in Fig. 11. The smooth continuous curve drawn through the points indicates that n begins to decrease from the constant value 1 in the neighborhood of $R=36$ ($\bar{u}=10.8$ cm. per sec.) and continues to decrease as R increases above this value.

We may now discuss the flow forms in their relations with n. As the mean velocity is increased so that R passes through the range $0 \leqslant R \leqslant 36$ the value of n remains constant and equal to 1. Thus the flow at $x=0$ in this range is laminar. The three-dimensional symmetrical flow (Fig. 3) gradually changes until at $R=25$ ($\bar{u}=7.5$ cm. per sec.) it transforms into the Blasius flow (Fig. 4) which persists until a Reynolds number of 36 is reached. During this transformation there has been no change of the initial velocity distribution. We may therefore consider the Blasius flow form to be the limiting case, as $\bar{u}$ increases, of a more general three-dimensional flow.

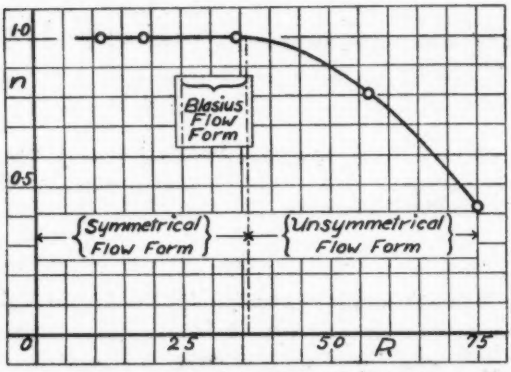

FIG. 11. *The variation of n with R showing the relation between the flow form and the type of motion near the walls of the channel at x=0.*

The work of Kármán (4) and Blasius (2) shows that for a turbulent flow $n=1/7$. It will be seen therefore that the transition from a laminar to a turbulent flow in the throat of the channel begins at $R=36$. But simultaneously with the beginning of this transition the symmetrical flow form begins to lose its symmetry and the flow tends to deviate toward one wall. This deviation increases as n decreases (Fig. 5) until at a value $R=70$ ($\bar{u}=21.0$ cm. per sec.) the flow breaks away from one wall only (Fig. 6). This flow form was found to persist with little change up to $R=75.1$ where, since $n=0.423$, the transition to the turbulent flow is still incomplete.

* *The Reynolds number used here is defined by the relation* $R = \frac{\rho w \bar{u}}{\mu}$, *where* w ($=0.5$ *cm.*) *is the width of the channel where the divergence begins,* $\bar{u}$ *is the mean velocity of flow calculated for the central horizontal plane of the channel at the line* $x = 0$, *and* ρ, μ *are respectively the density and viscosity of air at 23° C.*

In conclusion we may say: (1) the Blasius flow form is the limiting case, as $\bar{u}$ increases, of a more general three-dimensional flow and it exists in the range $25 \leqslant R \leqslant 36$; (2) the symmetrical Blasius flow form transforms, as R increases, to an unsymmetrical flow which breaks away from one wall only, the transition taking place over the range $36 \leqslant R \leqslant 70$; (3) at the point where the transition from the symmetrical to the unsymmetrical flow begins ($R = 36$), the flow near the walls at $x = 0$ ceases to be laminar and begins a transition to a turbulent flow which progresses as R increases.

Acknowledgments

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A DISCHARGE TUBE LIGHT SOURCE WITH CONTROL CHARACTERISTICS SIMILAR TO THOSE OF A THYRATRON[1]

By D. C. Rose[2]

Abstract

Discharge tubes having electrical characteristics similar to those of thyratrons have been used as flashing light sources for recording purposes. The tubes differ from ordinary thyratrons in that the space between the control electrode and anode has been increased, so that the light given out by the intense discharge in this space is available. Two different tubes have been constructed. In the first the anode was placed in a side tube connected to the main tube by a capillary. The discharge through the capillary was used as the light source. An additional electrode was used in the main tube to start the discharge. In the second tube the space between the control electrode and the anode was enclosed by a cylindrical shield having a slit cut in its side through which the light from the discharge passed. These tubes were used successfully for recording the instant that beams of light were interrupted by rifle bullets in a new velocity-measuring apparatus. The tubes have the advantage over usual discharge tube light sources that they are as easily controlled as a thyratron. Other applications of such tubes as light sources are discussed.

The thyratron, though developed primarily as a relay device, has also been found to have uses as a light source for stroboscopic and recording purposes. Robertson and Rogers (3) and Kluge (2) describe the use of thyratrons as light sources for stroboscopic work. In measuring the velocities of projectiles the author (4) has used the light from an FG 17 thyratron to record the time of their flight. The flashes of light from the thyratron, as it discharged a condenser, were recorded photographically, the flashes being produced when the bullet interrupted beams of light placed along the trajectory. Other applications, such as the photographic recording for high speed counters of various sorts or any work where short time intervals between phenomena must be known accurately, suggest themselves.

Thyratrons, as usually designed, have the great disadvantage that the most intense part of the discharge takes place in a region which is so nearly enclosed by the electrodes that very little light escapes and what does is usually unduly diffused throughout the tube. An extended or diffuse light source may be useful for stroboscopic work, but for the recording purposes such as that required in the measurement of projectile velocities (4), or other cases where short time intervals between irregularly spaced and non-repeatable phenomena are being measured, the light source should be very intense and concentrated in a small area approaching a line or point source.

The discharge tubes described in this paper have the above-mentioned properties combined with the ease of control of an ordinary thyratron. In principle they are designed like a thyratron but the electrodes and glass envelope are so arranged as to emit as much light as possible but to confine it to a narrow line source. Edgerton and Germeshausen (1) have used for

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Contribution from the Division of Physics, National Research Laboratories, Ottawa, Canada.

[2] *Physicist, National Research Laboratories, Ottawa.*

stroboscopic work a controlled mercury discharge tube which could be used for purposes similar to those for which the tubes built by the author were used, but their tube suffers from the disadvantage that a high voltage, and therefore considerable energy from the control circuit, is required to start the discharge.

Description of Tubes

First Tube

Fig. 1 shows a diagram of the first tube tried, the important dimensions being given. The anode of the tube was located in a side tube connected to the main tube by a capillary. The capillary tube was used as the light source. The cathode consisted of an oxide-coated nickel strip about 1.5 mm. wide, 0.1 mm. thick and about 60 mm. effective length. The oxide coating was prepared from barium carbonate applied by the candle method and sensitized in the usual way. It would emit sufficient electrons to carry a continuous current of almost two amperes, though of course the other electrodes would not stand this current for any length of time. A ring electrode was placed between the openings in the control electrode and the capillary tube, for the purpose of starting the discharge, so that unreasonably high voltages would not be required on the anode placed in the side tube far from the cathode and control electrode. If the ring electrode is used as an anode, the breakdown characteristics of the tube are similar to those of ordinary thyratrons of about the same size. The impedance of the capillary was such that 400 or 500 volts were required on the anode to start a visible discharge through it even when the main part of the tube was in a conducting state, *i.e.*, when current was flowing from the ring to the cathode.

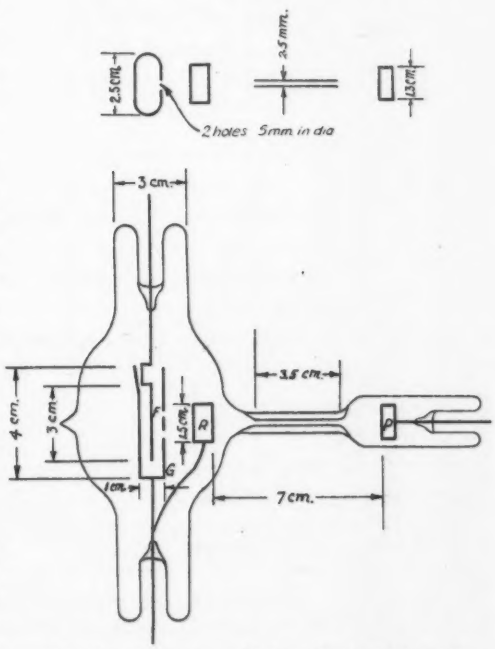

FIG. 1. *Arrangement of electrodes in the first tube.*

The tube was not designed for operation with continuous currents to the anode. If any appreciable current was made to flow continuously through the capillary tube it became dangerously hot. Hence for the most part its breakdown characteristics were studied while it was connected in a relaxation

oscillator circuit. The most satisfactory way to connect the ring to the power supply was that shown in the inset in Fig. 2. Under operating conditions the control electrode was maintained at a potential sufficiently negative that no discharge took place. Making it more positive than values given by the curves in Fig. 2 caused the tube to oscillate; or a short positive pulse on the control electrode would cause one discharge giving one flash of light. To obtain these curves the leads marked control were short-circuited and a voltmeter was connected from the cathode to the control electrode. The plate and ring voltages, as shown in Fig. 2, were calculated from the drop in the resistances in the plate circuit with the tube in a non-conducting state, the voltmeter being connected across the power input as indicated. The curves indicate the points at which the grid was just sufficiently positive to allow oscillation. Observations could be repeated to within 0.1 volt. The temperatures indicated are the temperatures of the air in a heated box surrounding the tube. Comparison of the curves in Fig. 2 with characteristic curves obtained when the ring was used as an anode shows that, over the range of voltages used, the discharge was controlled by the ring, and grid or control electrode. The anode voltages used were not high enough to make the tube conduct unless the discharge was started in the main part of the tube by appropriate voltages on the ring and control electrodes. In fact the ring would serve equally well for a control electrode but of course was not nearly as sensitive as the grid.

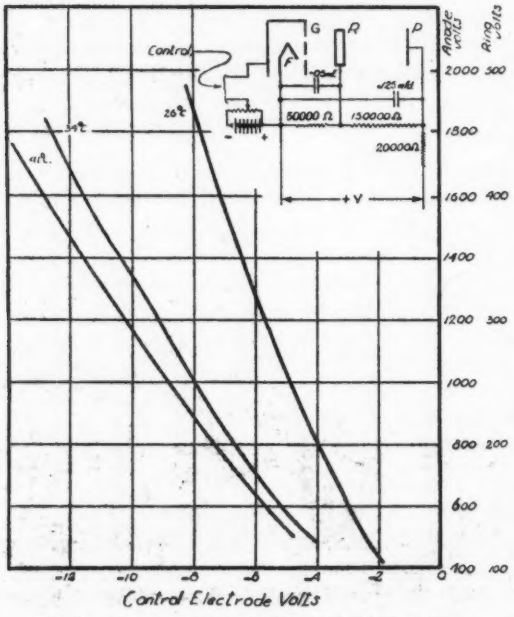

FIG. 2. *Discharge characteristics and circuit diagram for the first tube.*

As previously mentioned (4) the tube was used as part of a recording device for measuring the velocities of projectiles. The projectile (a rifle bullet) interrupted successively beams of light shining on photoelectric cells placed along the trajectory. The potential pulses, caused by the momentary change in light intensity, after being amplified, were impressed on the grid of the light-emitting thyratron. Fig. 3 shows a record obtained with the tube described above. The time marker on this record was made by an Einthoven galvanometer excited by an alternating current of 1000 cycles from a valve-main-

tained tuning fork. Only the peaks of the galvanometer fibre oscillations were included on the record. Under more complete working conditions the time record would be obtained from another similar tube flashing 1000 to 500 times per second and controlled by the 1000 cycle voltage. As only one tube of this type was constructed the time record and bullet record of the same type could not be taken simultaneously. This tube would not oscillate at 1000 cycles per second with the values of resistances and condensers used, and it is doubtful whether it could be made to oscillate at that frequency without cutting down the light intensity considerably. However it oscillated steadily at 500, $333\frac{1}{3}$ or 250 cycles per second when the 1000 cycle control was used, the flashing frequency depending on the grid bias.

A spectroscopic analysis of the light emitted from the capillary was not made though it would be an interesting study, as the color of the light appeared to be different from that of the light from an ordinary mercury discharge. The light appeared distinctly more yellow as though the yellow and green lines were enhanced at the expense of the blue and violet parts of the mercury spectrum. Bright yellow specks also appeared on the walls of the capillary as though minute points were made incandescent. One would expect that the number of excited atoms per unit volume in the capillary tube would be higher than in a less constricted discharge, owing to the high electron density in the constricted tube. However, until a more quantitative study of the light emitted at different pressures and current densities is made, it is difficult to say anything about the state of excitation of the mercury in the capillary tube. When the tube was used for recording, the anode voltage (+V, Fig. 2) was usually about 1500.

The period of the flash was extremely short, as shown by the line on the record. The lens system of the camera consisted of one cylindrical lens having its axis parallel to that of the capillary tube. The lens had an aperture (normal to its axis) of about 10 mm. and a focal length of 15 mm. The tube was placed about 25 cm. from the lens and the line image of the capillary was focused on the film in the rotating drum camera. With film speeds of about 5 mm. in 1/1000 second the record on the film did not appear any wider than the stationary

FIG. 3. *Record showing flashes of light from the first tube and time marker in apparatus for measuring projectile velocity.*

image of the capillary tube, and it was sharp on both edges, indicating that the time of discharge was not greater than a few micro seconds, probably considerably less than 10. Eastman Verichrome films were used in taking these records.

Second Tube

The second tube was designed with a view to decreasing the cathode to anode impedance so that lower anode voltages could be used. It was only partially satisfactory in this respect, but it has advantages over the first in that it gives more light, or more actinic light, than the first. A diagram of the tube is shown in Fig. 4. The arrangement is very similar to that of the

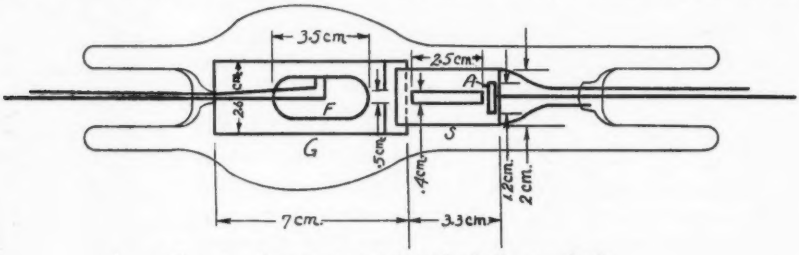

FIG. 4. *Arrangement of electrodes in the second tube.*

FG 17 thyratron, except that the distance between the grid and the anode was much greater and the space between them was enclosed by a cylindrical shield. This shield was slit on one side so that a narrow band of light was emitted. The shield was connected to the anode through a resistance of 25,000 ohms as shown in the inset circuit diagram in Fig. 5. To prevent the shield from carrying any of the discharge it should have been connected to the cathode. However, when it was connected to the cathode, the shielding effect was so great that the tube could not be made to discharge to the anode with the highest voltage available, namely, 2400 volts; and, with the shield connected as shown in Fig. 5, it appeared to carry a negligible part of the discharge and the tube worked very satisfactorily at 1000 volts. The cathode was prepared in a manner similar to that

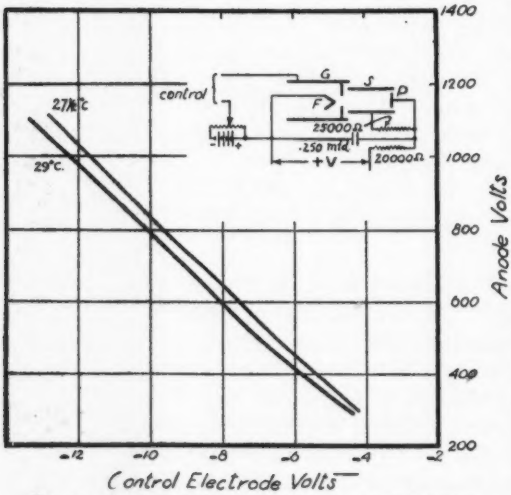

FIG. 5. *Discharge characteristics and circuit diagram for the second tube.*

in which the cathode of the first tube was prepared, but the electron-emitting area was about double. The curves in Fig. 5 represent the breakdown characteristics of this tube.

Fig. 6 shows records taken with the second tube used both to record interruptions of a light beam as in Fig. 3 and as a time marker emitting 500 flashes per second. Actually, the rifle range was not in operating condition when this tube was being tested, so a light beam was interrupted approximately 60 times per second by a cross arm bolted to the pulley of an electric motor. The light beam excited a photoelectric cell and the current pulses caused by the interruptions were made to control the same amplifier used in the projectile-velocity measurements. The period of the flash appears to be just as short as in the case of the first tube. The intensity of the light was considerably greater than that emitted by the first tube, but the two should not be compared as the energy of the discharge was considerably greater in the second case. Further, the discharge appeared different. In the second tube the discharge appeared to be the color of that in an ordinary thyratron.

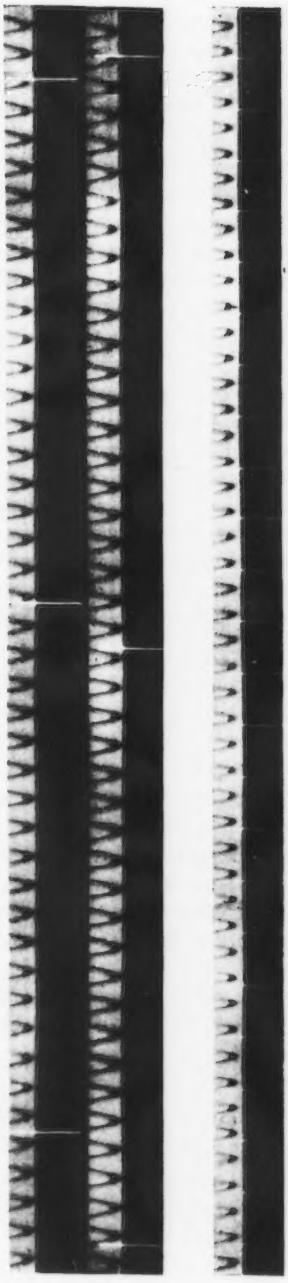

FIG. 6 *Record showing flashes of light from the second tube, used in the apparatus for measuring projectile velocity, and also used as a time marker giving 500 flashes per second.*

Discussion

To make an efficient light source in a discharge tube, obviously a maximum number of atoms of the gas or vapor should be maintained in an ionized or excited state. In the case of a mercury arc where the hot pool of mercury at the cathode acts as an unlimited source of ions, the light intensity is limited only by the amount of energy that can be put into the discharge. However, such is not the case with tubes functioning as thyratrons where the main part of the discharge is carried by electrons emitted by a hot cathode. In fact, in using the thyratron as a relay device, satisfactory operation is achieved when the ionization is just sufficient to neutralize the space charge.

The amount of current carried by the positive ions in a thyratron is supposed to be small. When the tube is in a conducting state, voltages across the tube must be kept sufficiently low that positive ion bombardment of the cathode does not lower its efficiency as a source of electrons. The desired conditions for the thyratron as a relay and as a light source are opposite extremes, since in the ideal tube used as a relay the energy loss should be a minimum, while, as a light source, as much energy as possible should be transferred into light.

The amount of light emitted by such a tube will depend on the electron-emitting power of the filament, the pressure of the mercury vapor, the voltage across the tube when in a conducting state if used on continuous discharge, and both voltage and capacity of the condenser if used in a relaxation oscillator circuit. It will also depend on the nature and geometric arrangement of the electrodes, as is indicated by the different appearance of the light emitted by the two tubes discussed above. The size of the tube, arrangement of electrodes, etc., will be fixed to some extent by the purpose for which the tube is required, but to find the conditions for maximum light emission a careful spectroscopic and photometric study of the variation in the light intensity as a function of the above variables should be made. Like most electric lights the characteristics are probably such that the life of the tube will be shortened as its efficiency as a light source is increased, but experience with the two tubes constructed indicate that a reasonable compromise between efficiency and long life should be obtainable.

When the best conditions have been found, there is no reason why large tubes should not be built to give very intense light flashes for stroboscopic work, motion picture photography or any purpose where an easily controlled flashing lamp is desired. The part of the tube carrying the discharge could be of almost any shape.

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REVIEWS AND NOTES

Two Methods of Use when Dealing with Embedded Tissues

Several methods are in use for making temporary containers for liquid paraffin wax while embedding tissues, but most of them suffer from disadvantages in the matter of slow cooling, and in the amount of time expended in making special sizes.

The writer has obviated these difficulties in his own work by the use of hollow blocks of ice, made as follows.

Making Hollow Blocks of Ice

If a considerable number of blocks are required, and if the size to be used is appropriate, blocks may be made by filling the ice trays of a refrigerator with water, and allowing the water to chill until a substantial wall is built up around each "cube" and then pouring out the unfrozen centres. These hollow blocks will keep indefinitely in the refrigerator.

If blocks of special size are required, or if only a single one is needed, and none are on hand in the refrigerator, they may be made by melting out the centre of a piece of ice of appropriate size by means of a fine stream of hot water from an ordinary wash bottle, or even from the hot water tap.

Embedding in Hollow Blocks of Ice.

When embedding in these ice blocks, no special precautions need be taken, except to see that the paraffin is not so cool that it sets throughout its volume immediately on pouring into the ice. It will be found that a thick wall forms around the paraffin on pouring, and this provides a convenient floor for orienting the tissues. The cooling is extremely rapid, and as a result, the paraffin is free from all traces of crystallization, and cuts very well. Moreover, the blocks of paraffin separate cleanly and easily from the ice, owing to the melting of the latter. Of course, as soon as a skin forms over the paraffin, the whole should be thrown into ice water to hasten cooling still further.

Separating Portions of a Paraffin Block

The usual method of separating portions of a block of paraffin containing embedded tissues is to score the block with some sharp instrument, and then attempt to break it along the scored lines. This is not always successful, as the block may break along some other line, ruining tissues in the line of fracture.

This difficulty may be obviated as follows: a thin wire, such as the *E* string of a mandolin, or one strand of a woven-steel picture wire, is attached at one end to a convenient nail on the laboratory wall, and the other end allowed to hang free.

When it is desired to cut a piece out of a block, the wire is stretched tight horizontally, and the paraffin block rubbed to and fro along it so that the wire saws its way through the wax. The cut may be guided in any direction, depending on how the paraffin block is held. In this way, pieces may be cut from crowded blocks with no risk of damage to any part of the embedded tissues.

JOHN STANLEY

DEPARTMENT OF BIOLOGY,
QUEEN'S UNIVERSITY,
KINGSTON, ONTARIO,
CANADA.

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